

FIG. 1

PhenoSense™ HIV Resistance Test Vector.

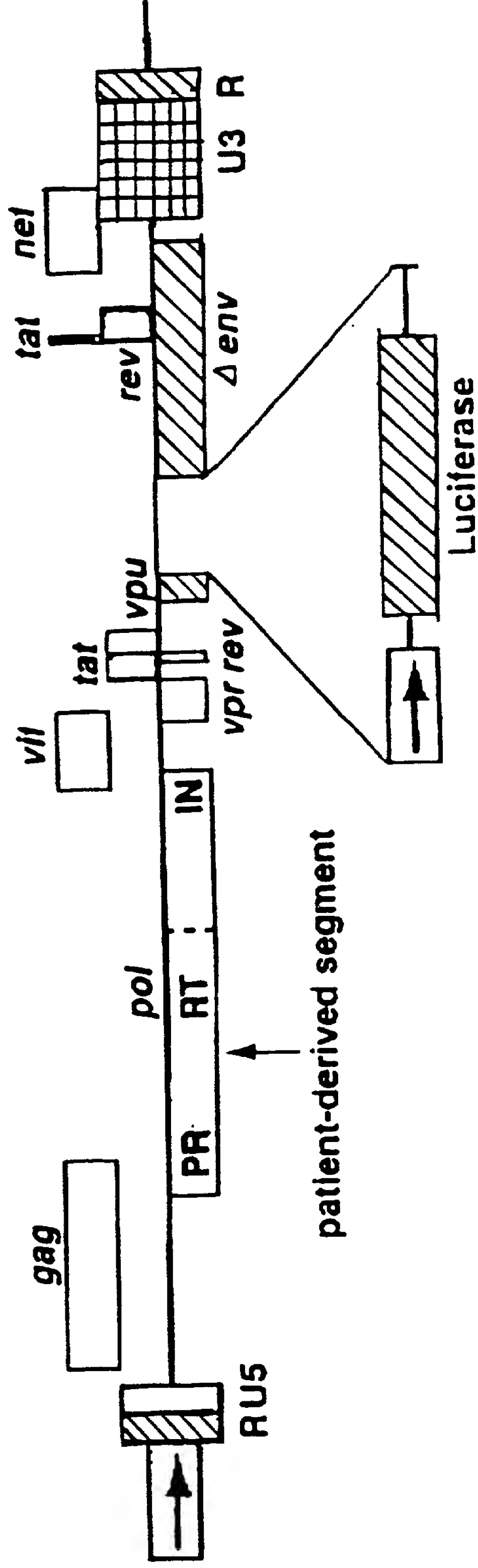
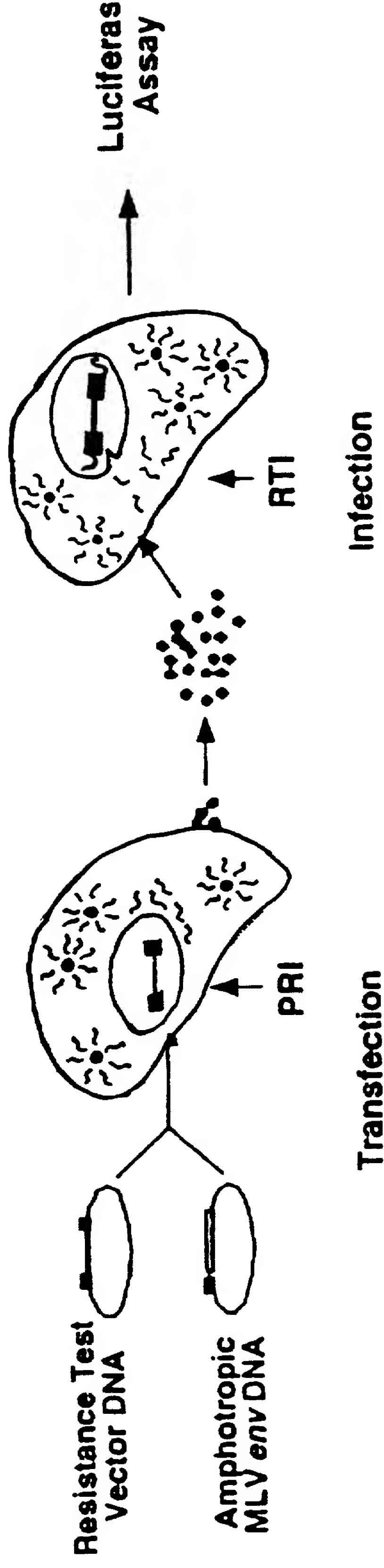


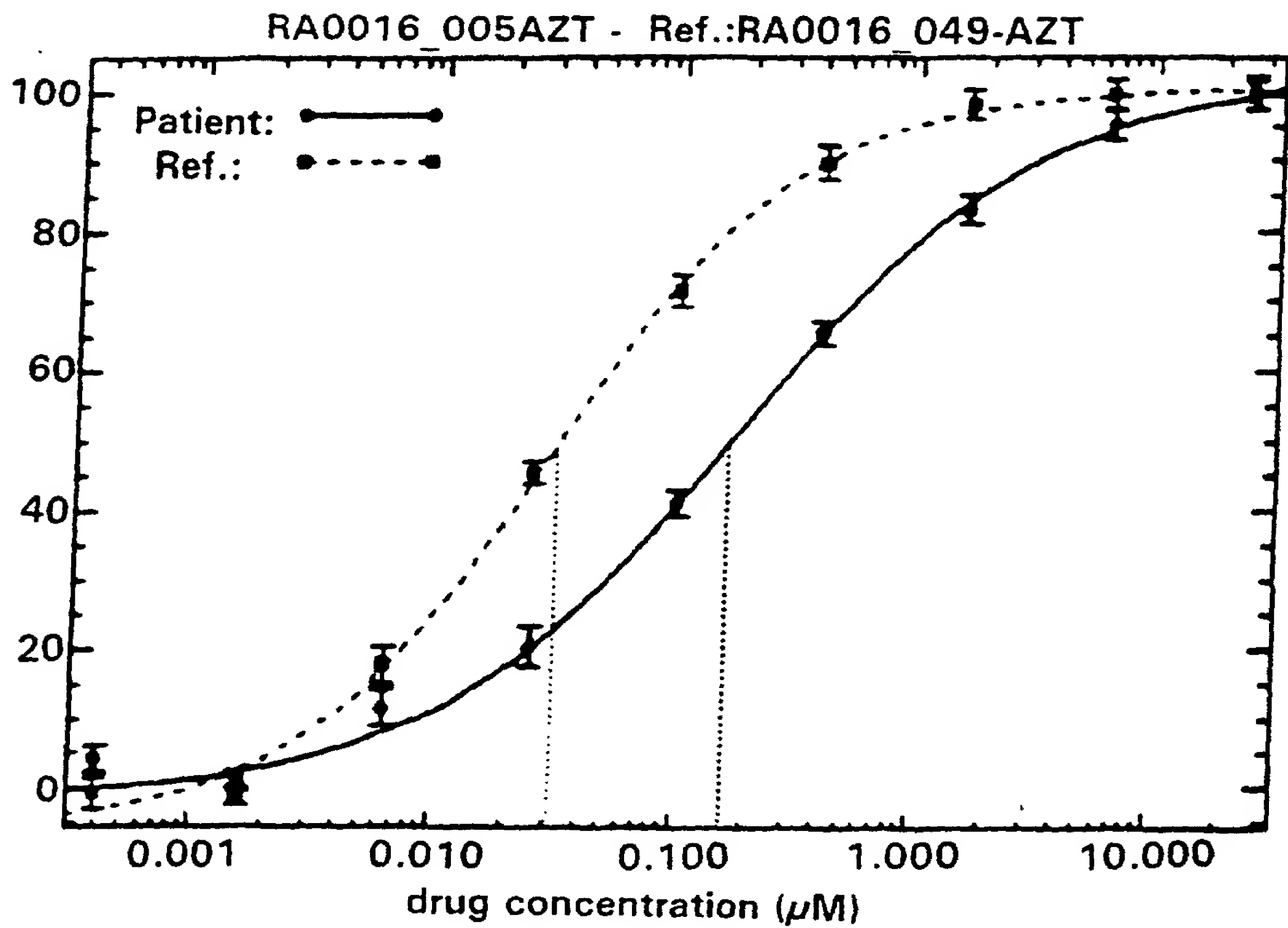
FIG. 2

PhenoSense™ HIV Schematic Diagram.



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FIG. 3A NRTI - AZT

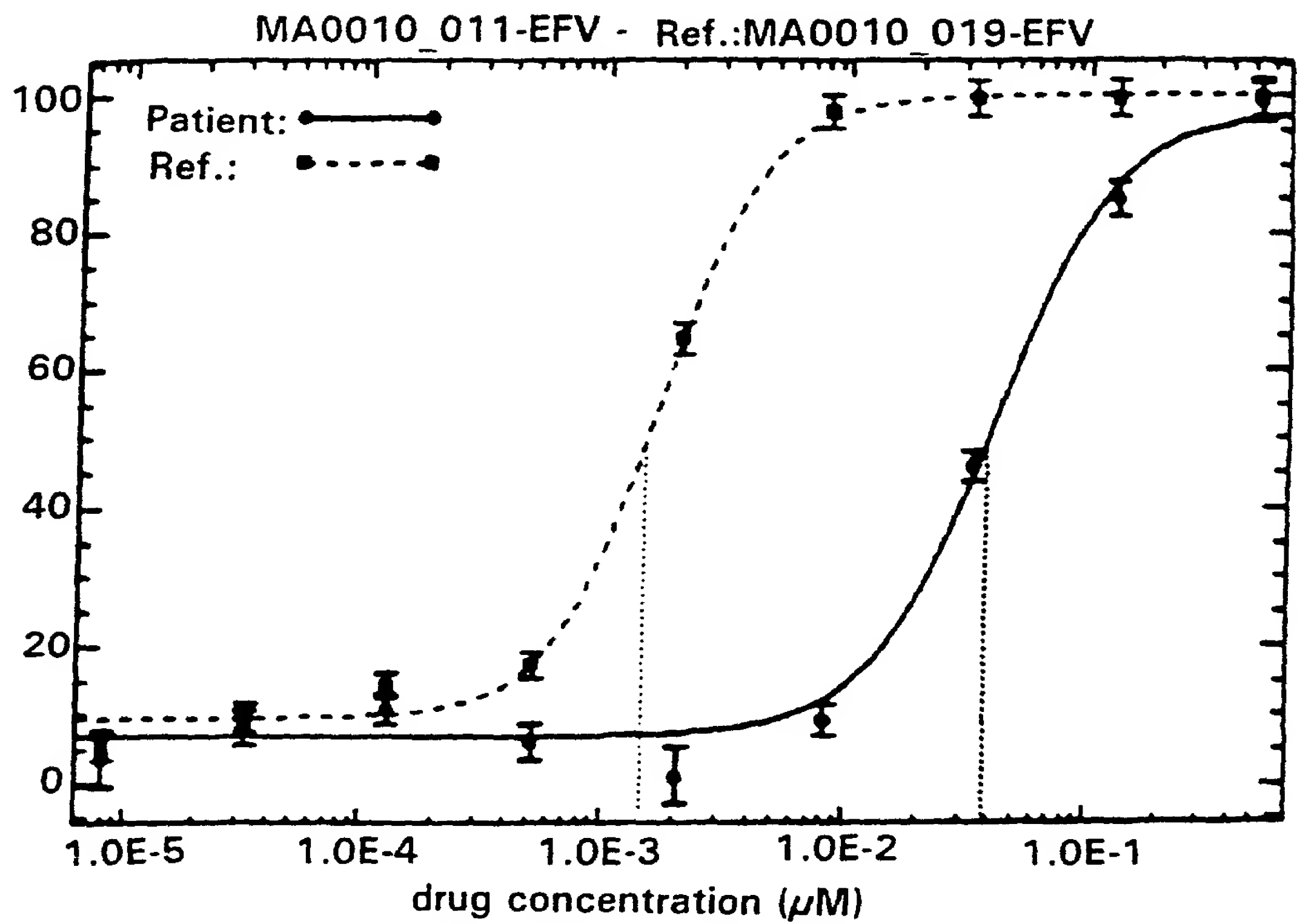


AZT-Control	$\text{IC}_{50} = 0.032$
AZT-Patient	$\text{IC}_{50} = 0.170$ (5.2-fold)

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FIG. 3B

NNRTI - Efavirenz

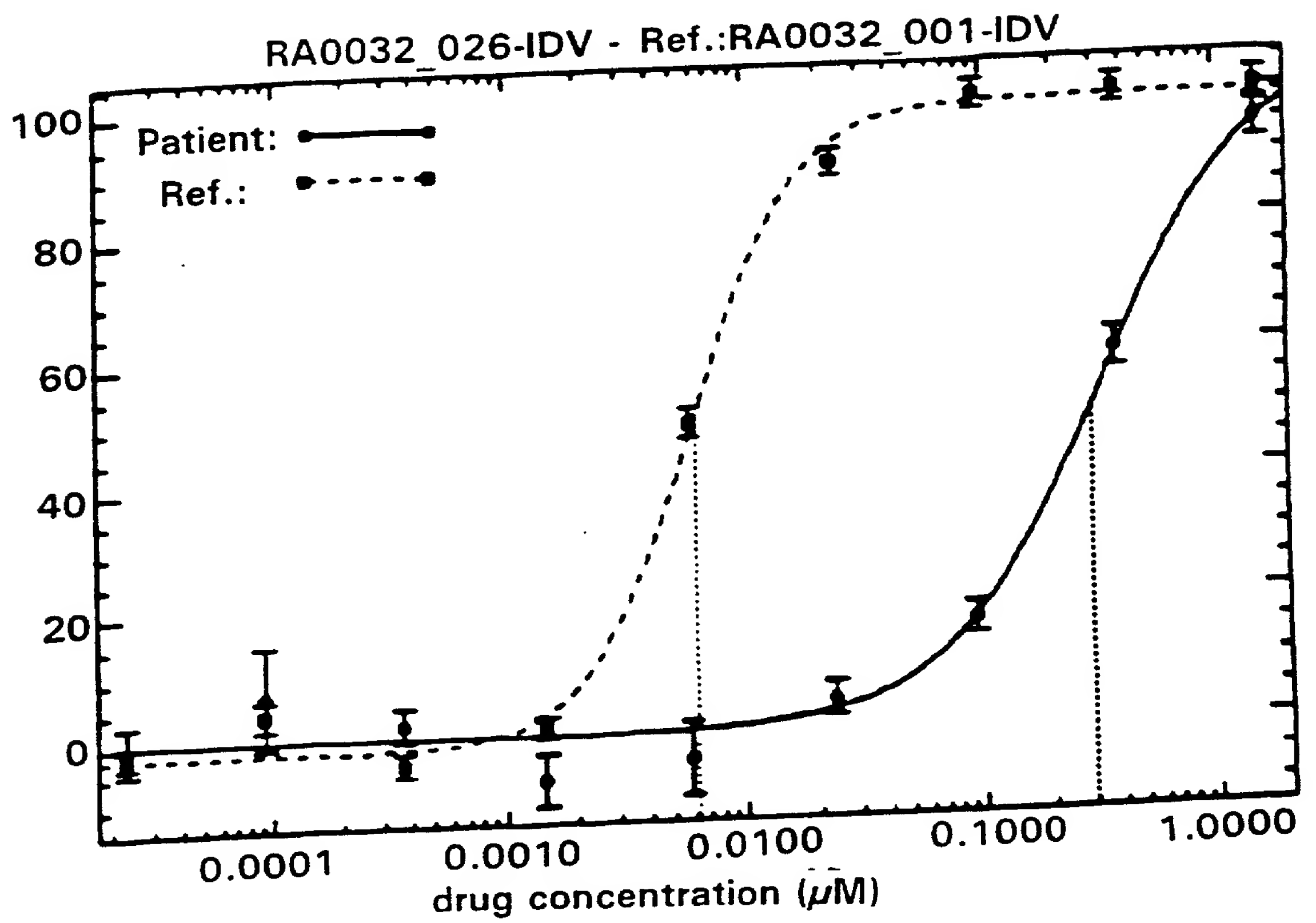


EFV-Control
EFV-Patient

$\text{IC}_{50} = 0.0015$
 $\text{IC}_{50} = 0.0380$ (25.6-fold)

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FIG. 3C PRI - Indinavir



IDV-Control
IDV-Patient

$\text{IC}_{50} = 0.0062$
 $\text{IC}_{50} = 0.2935$ (47.4-fold)

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FIG. 4A SQV

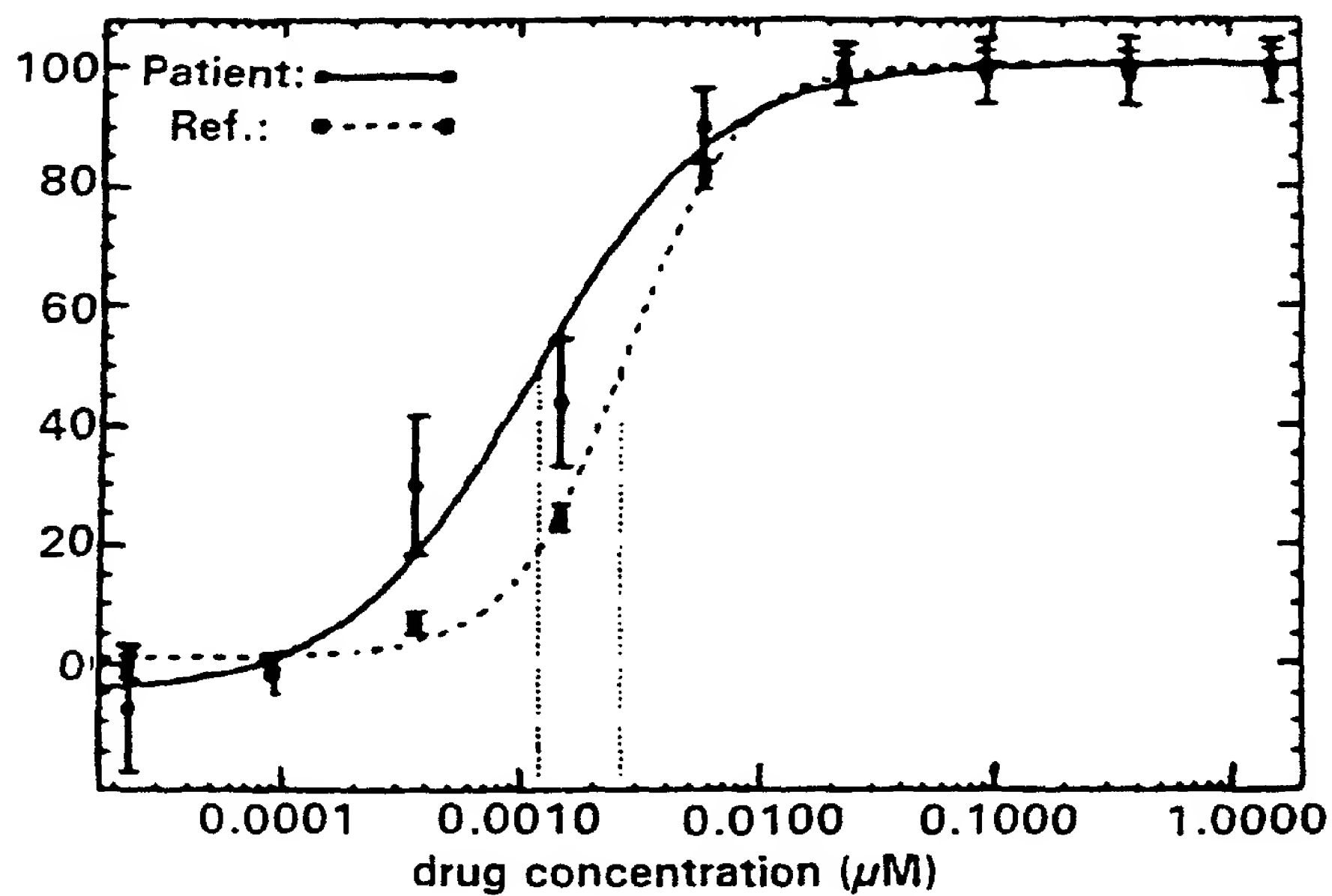
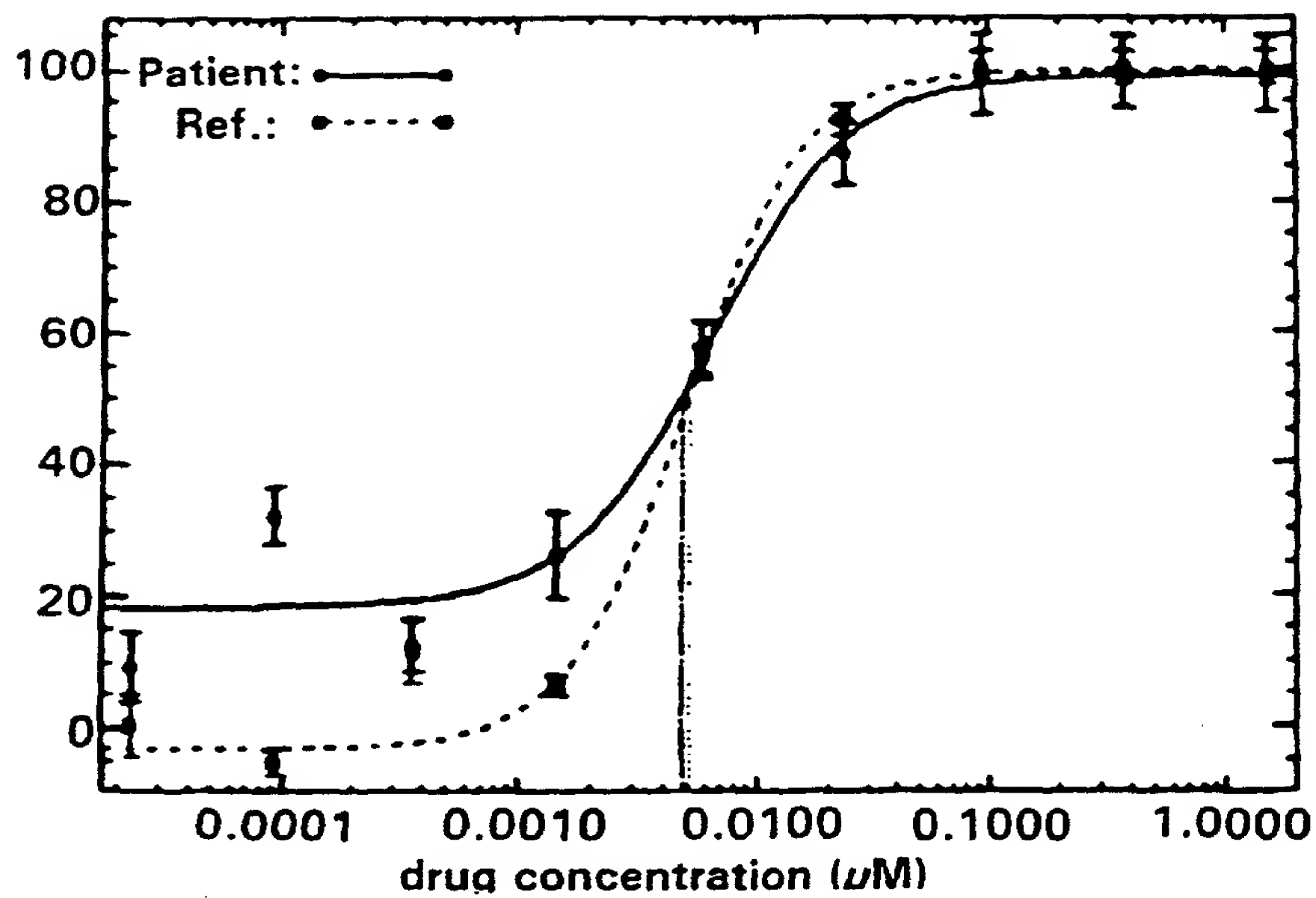


FIG. 4B IDV



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FIG. 4C RTV

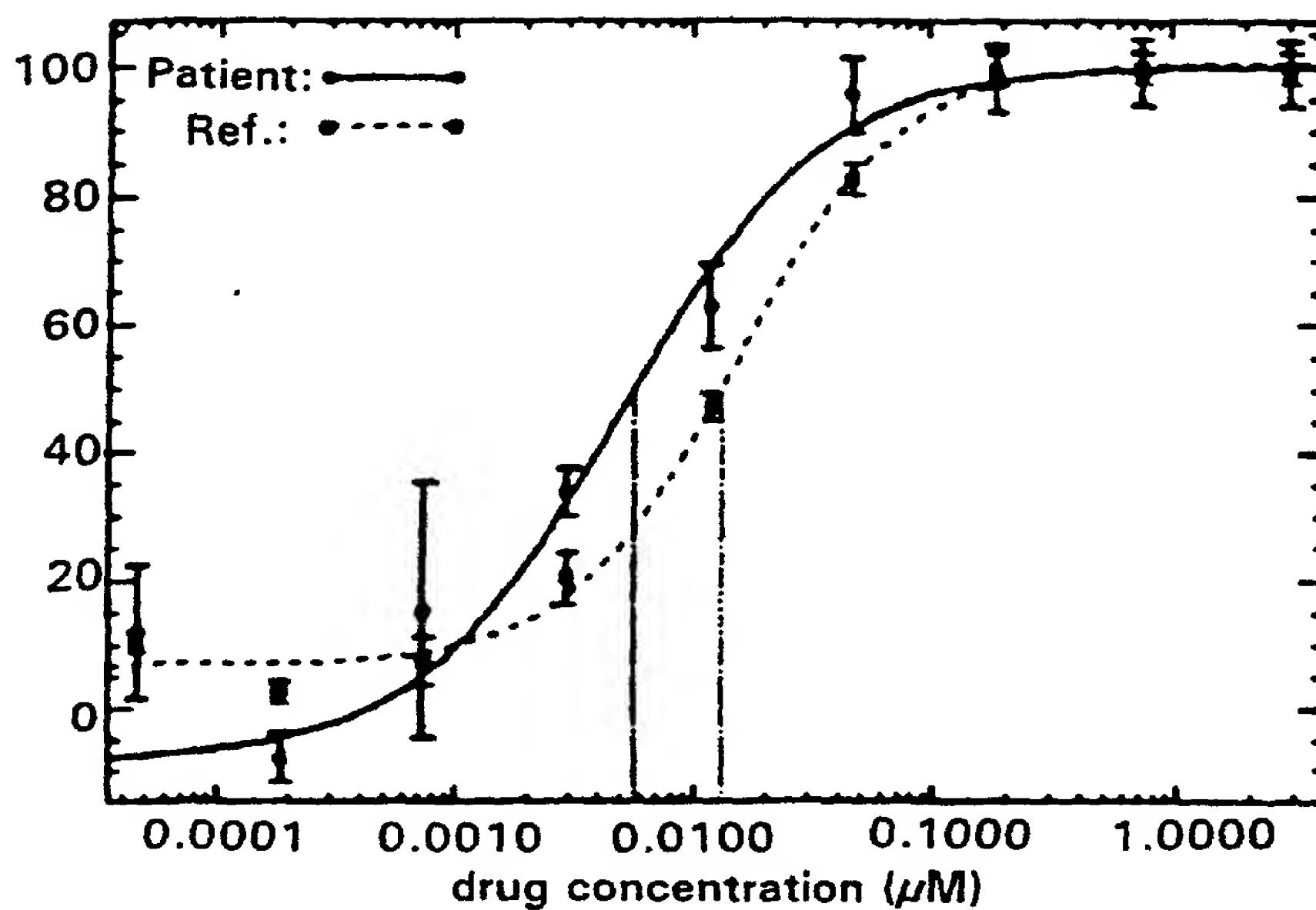
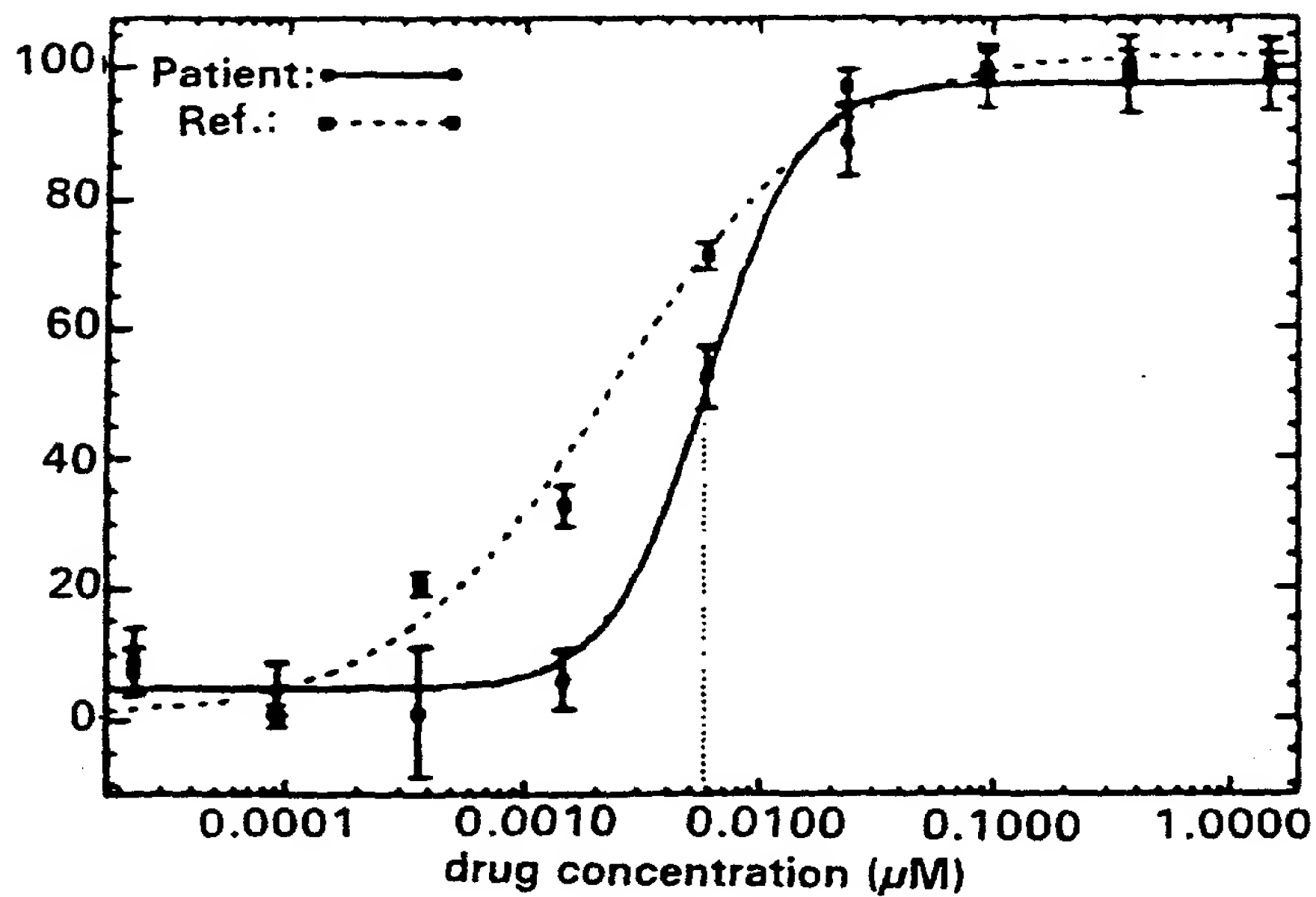


FIG. 4D NFV



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FIG. 4E AMP

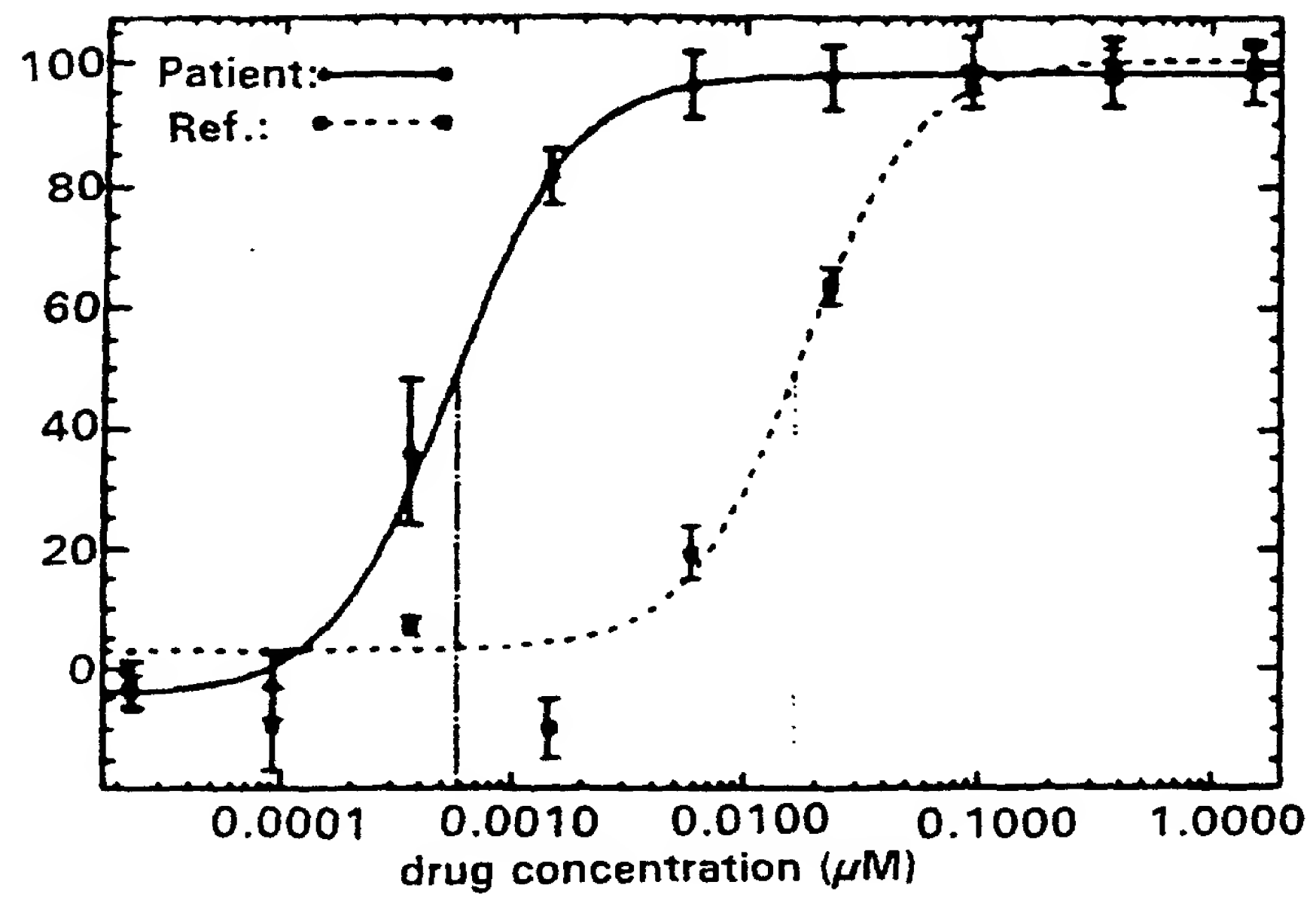
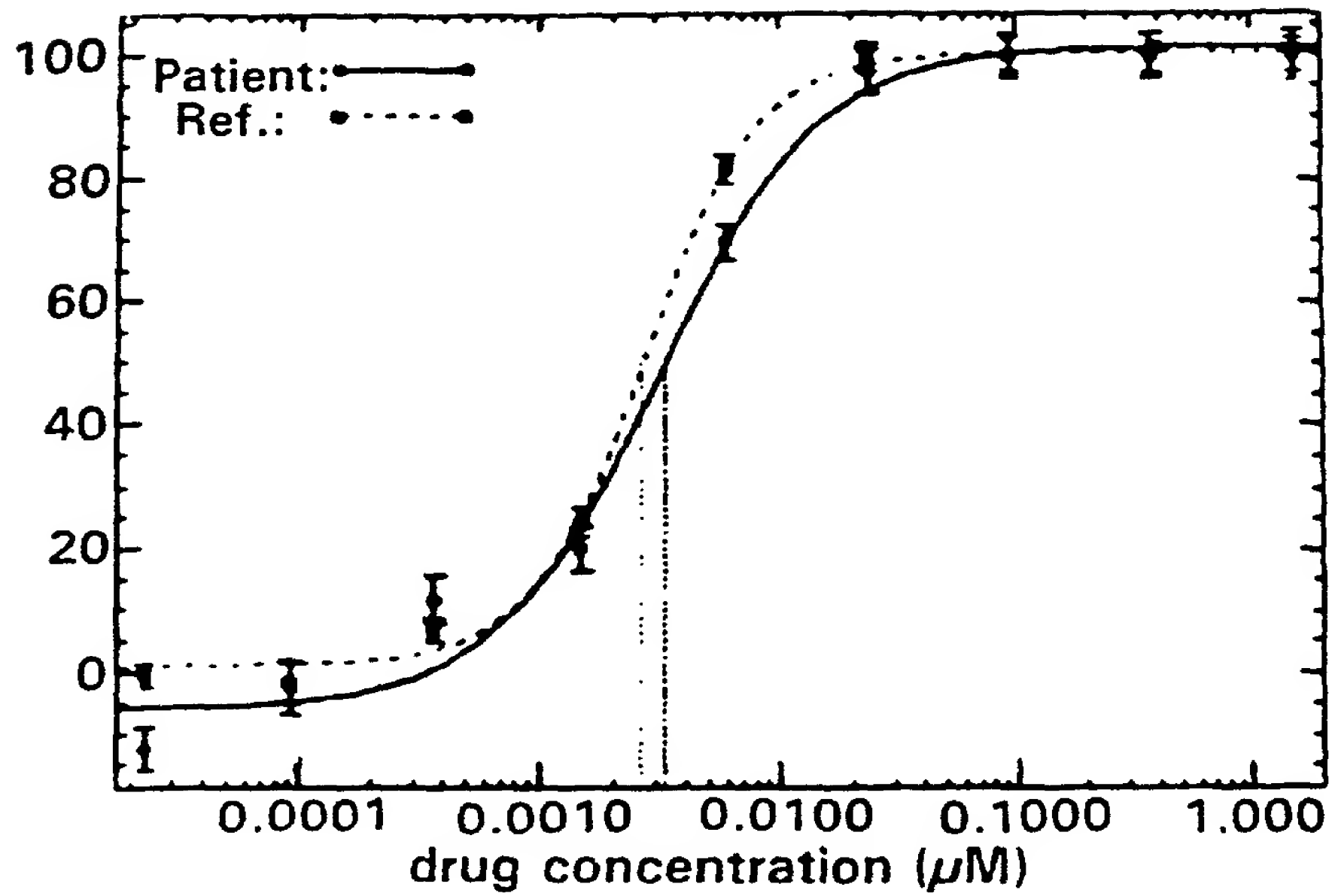
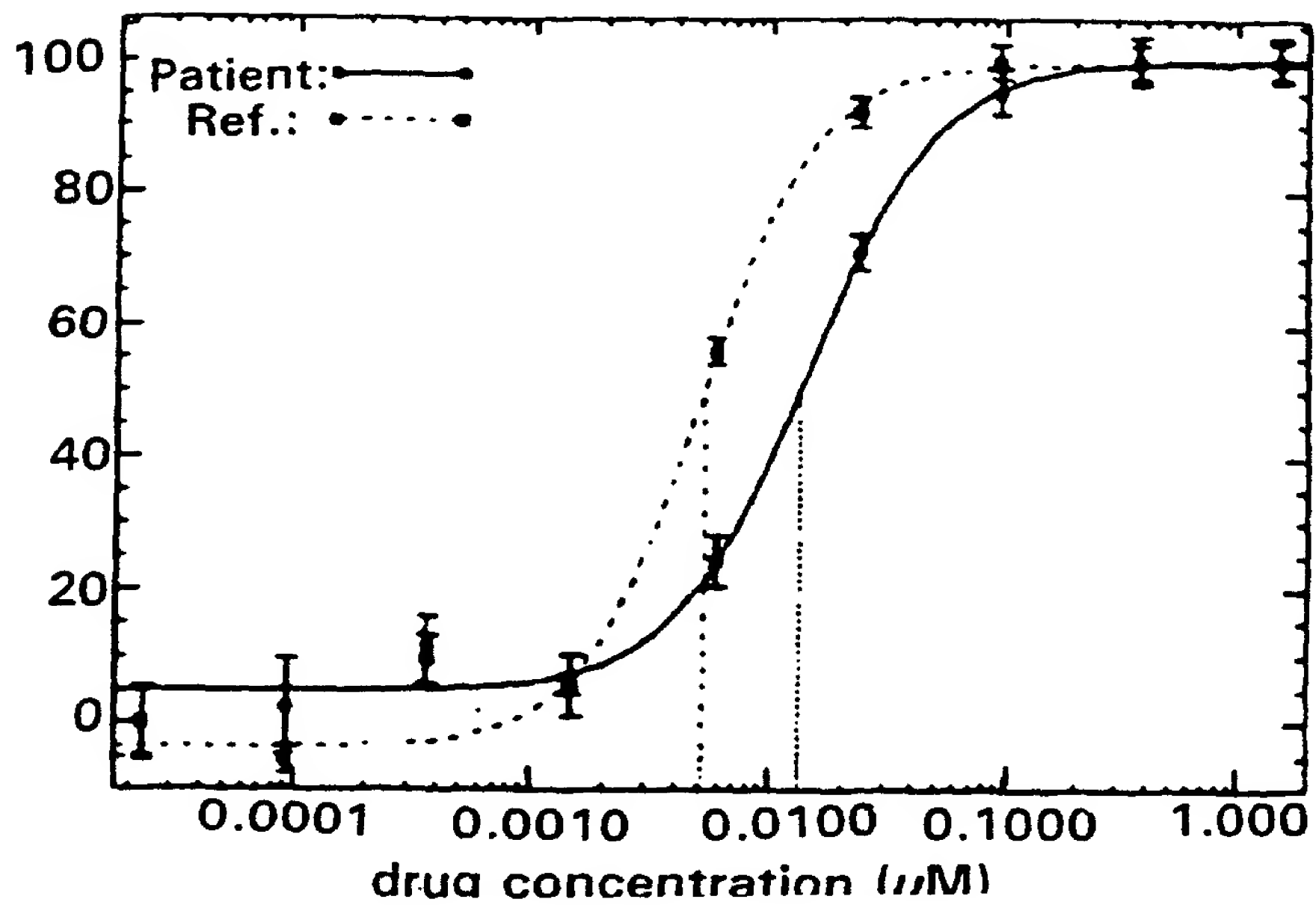


FIG. 5A SQV**FIG. 5B IDV**

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FIG. 5C RTV

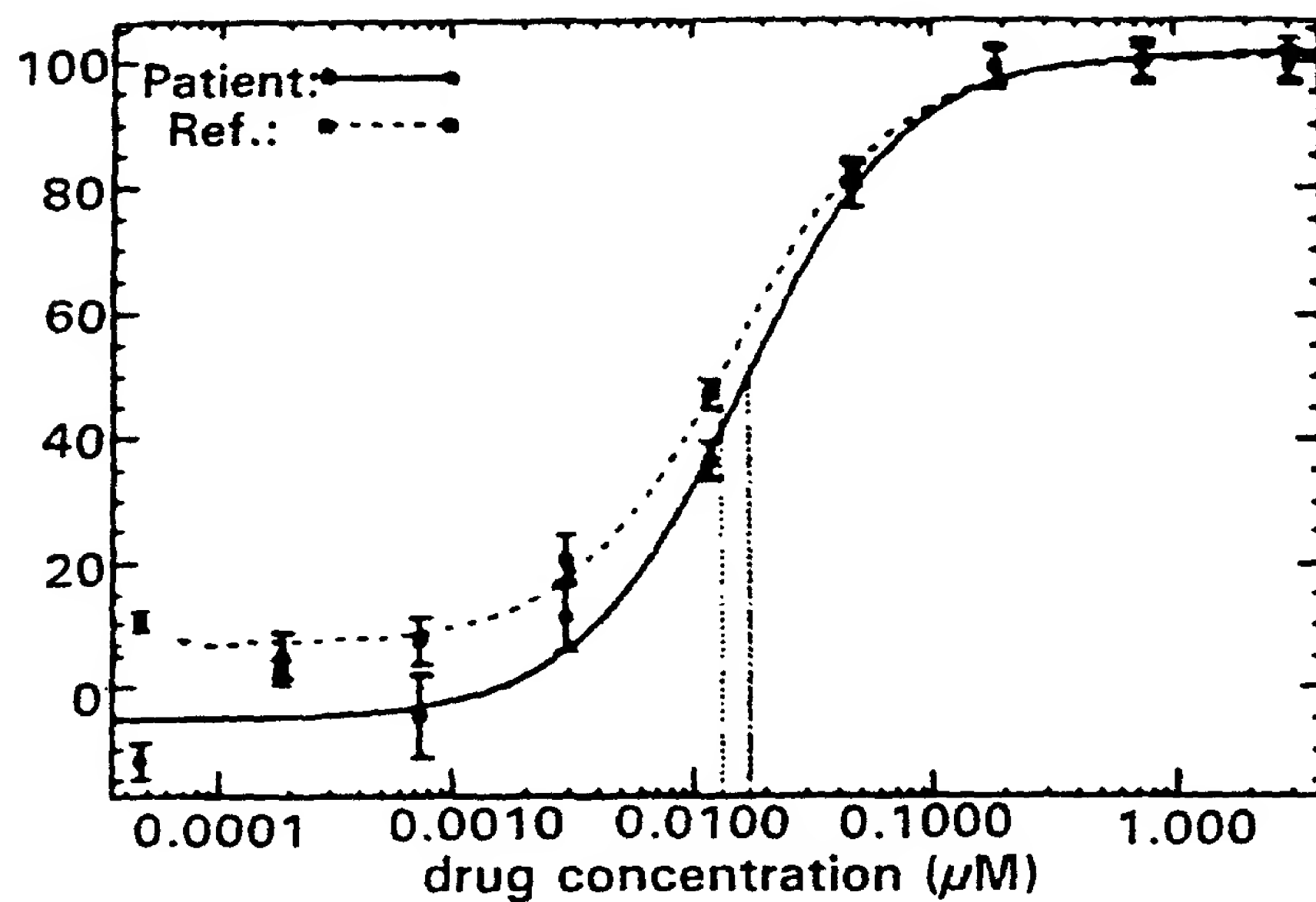
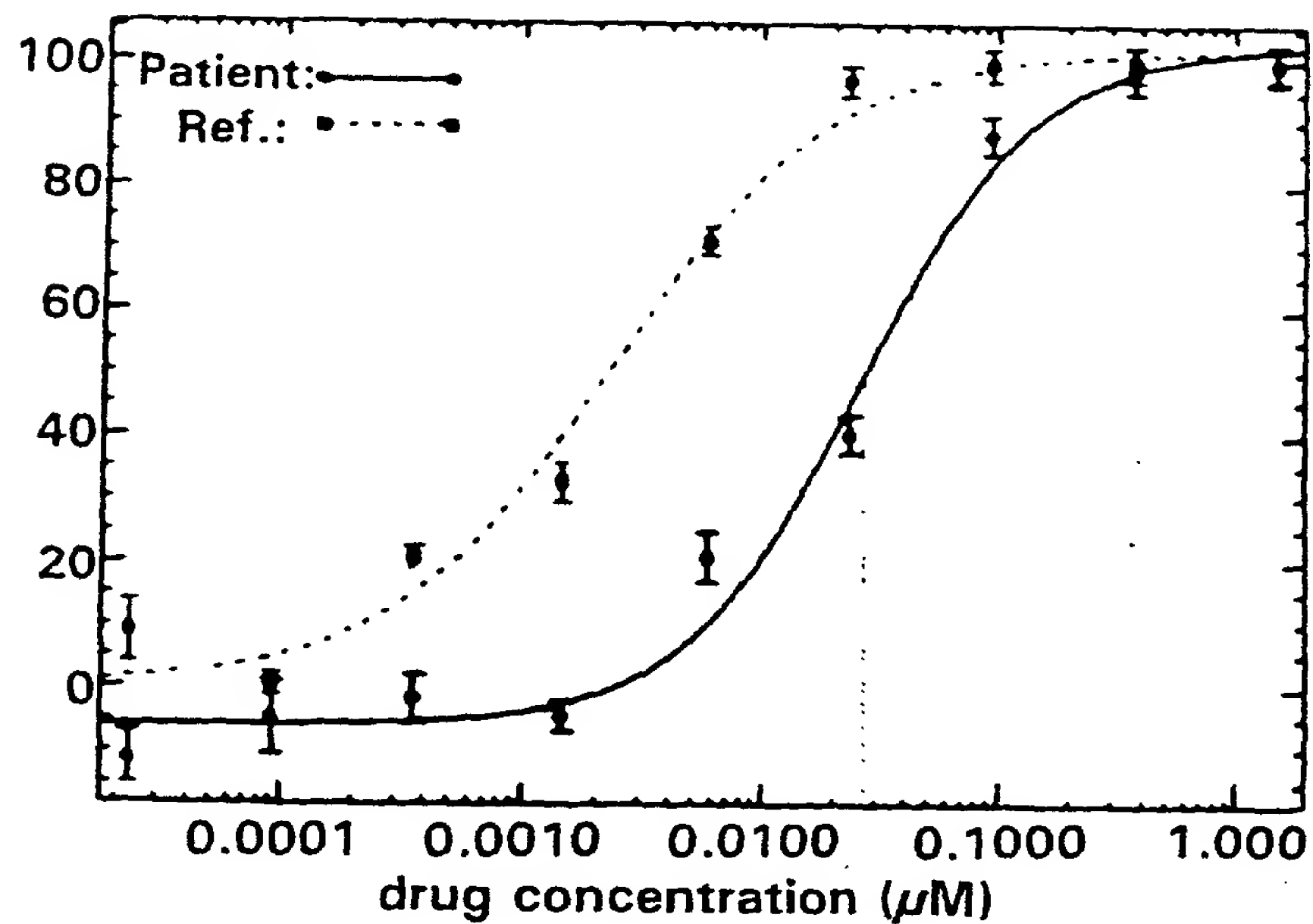


FIG. 5D NFV



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FIG. 5E AMP

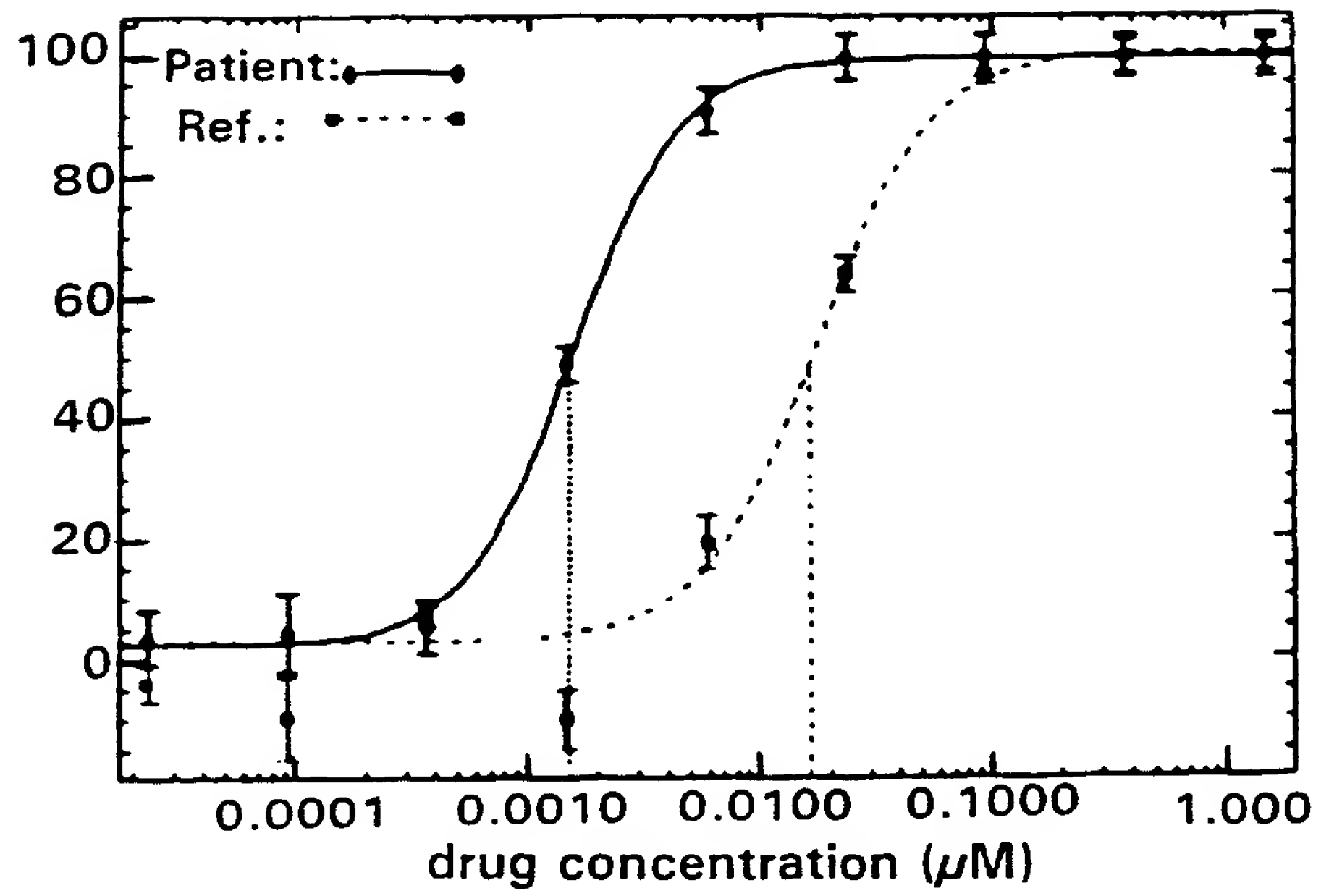


Figure A: Fitness Assay

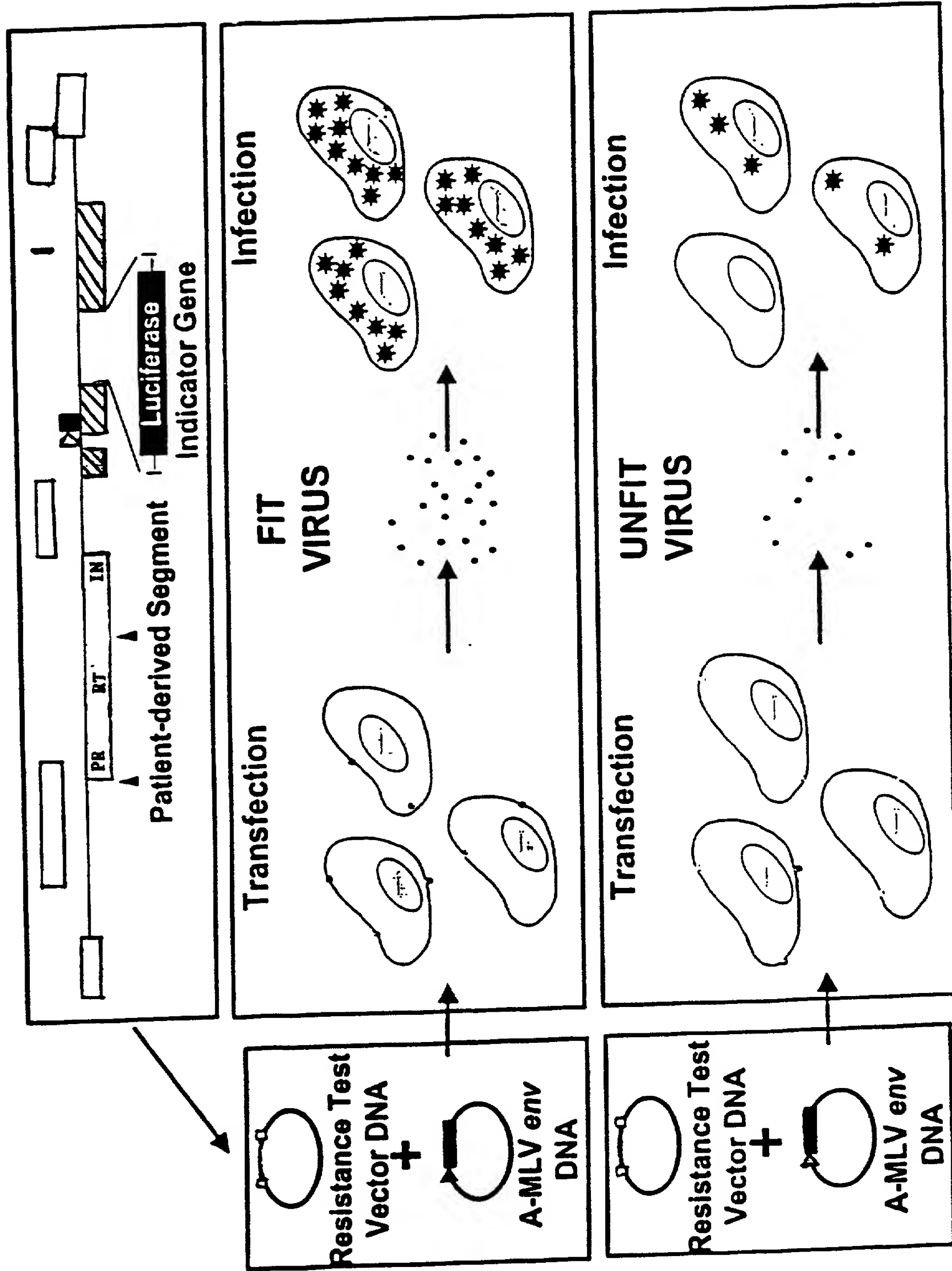


Figure B: Luciferase Activity in Infected Cells

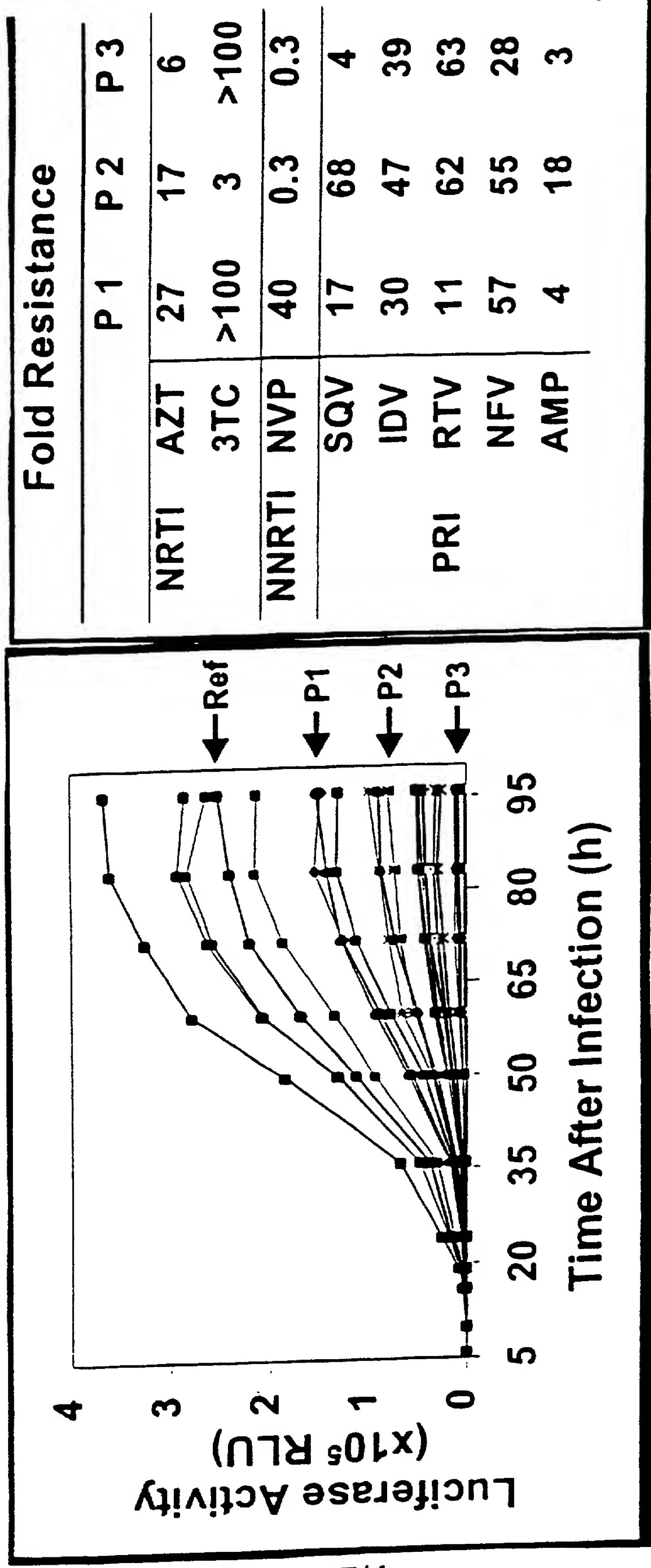
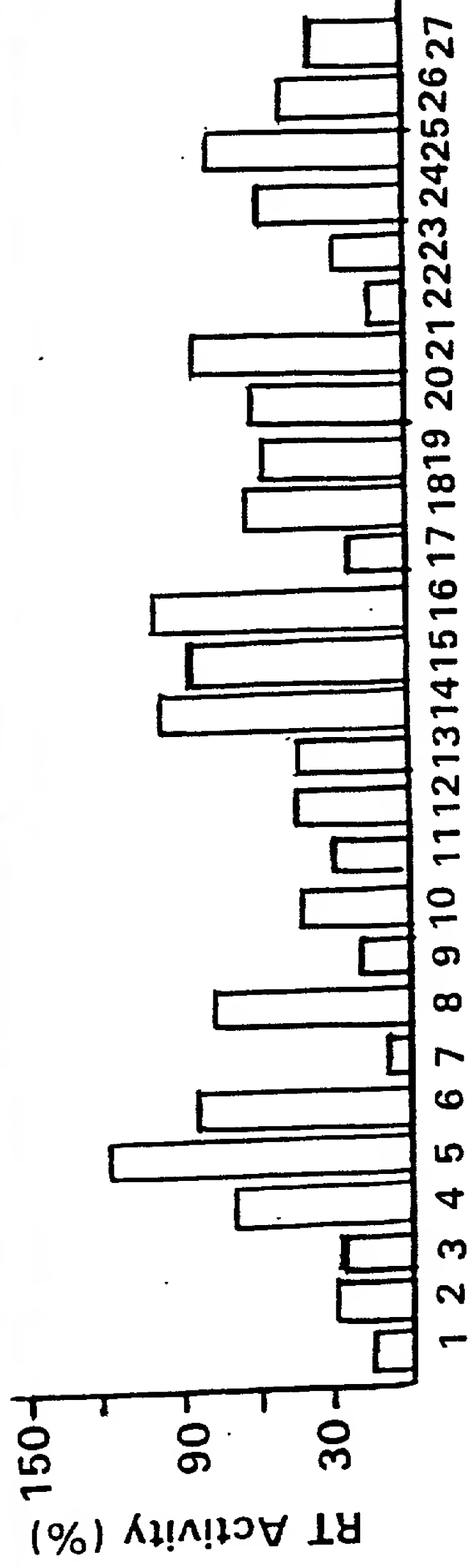
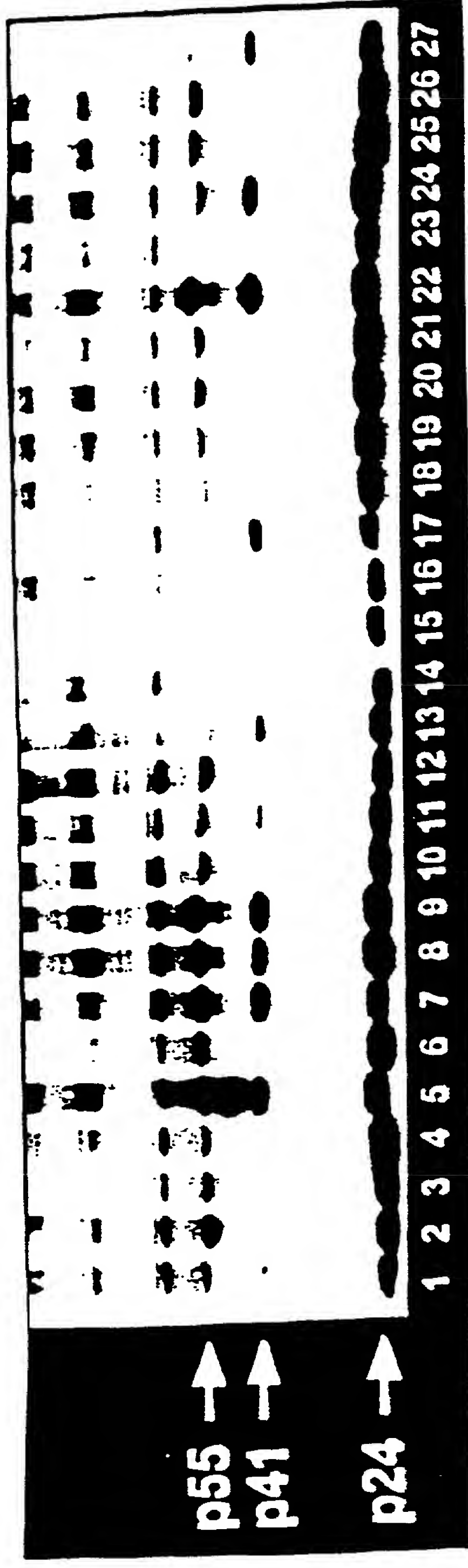
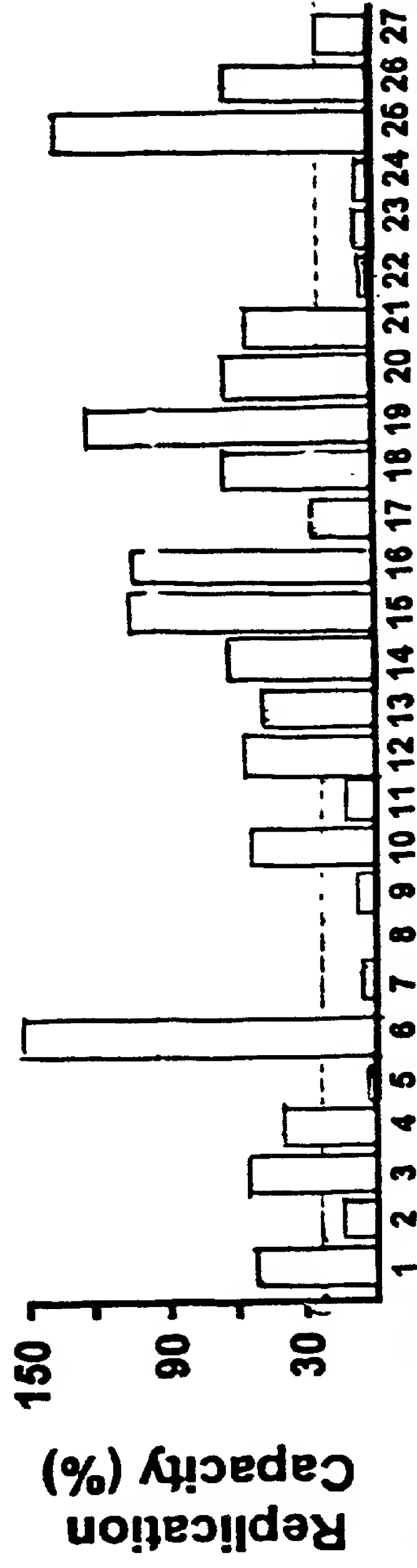
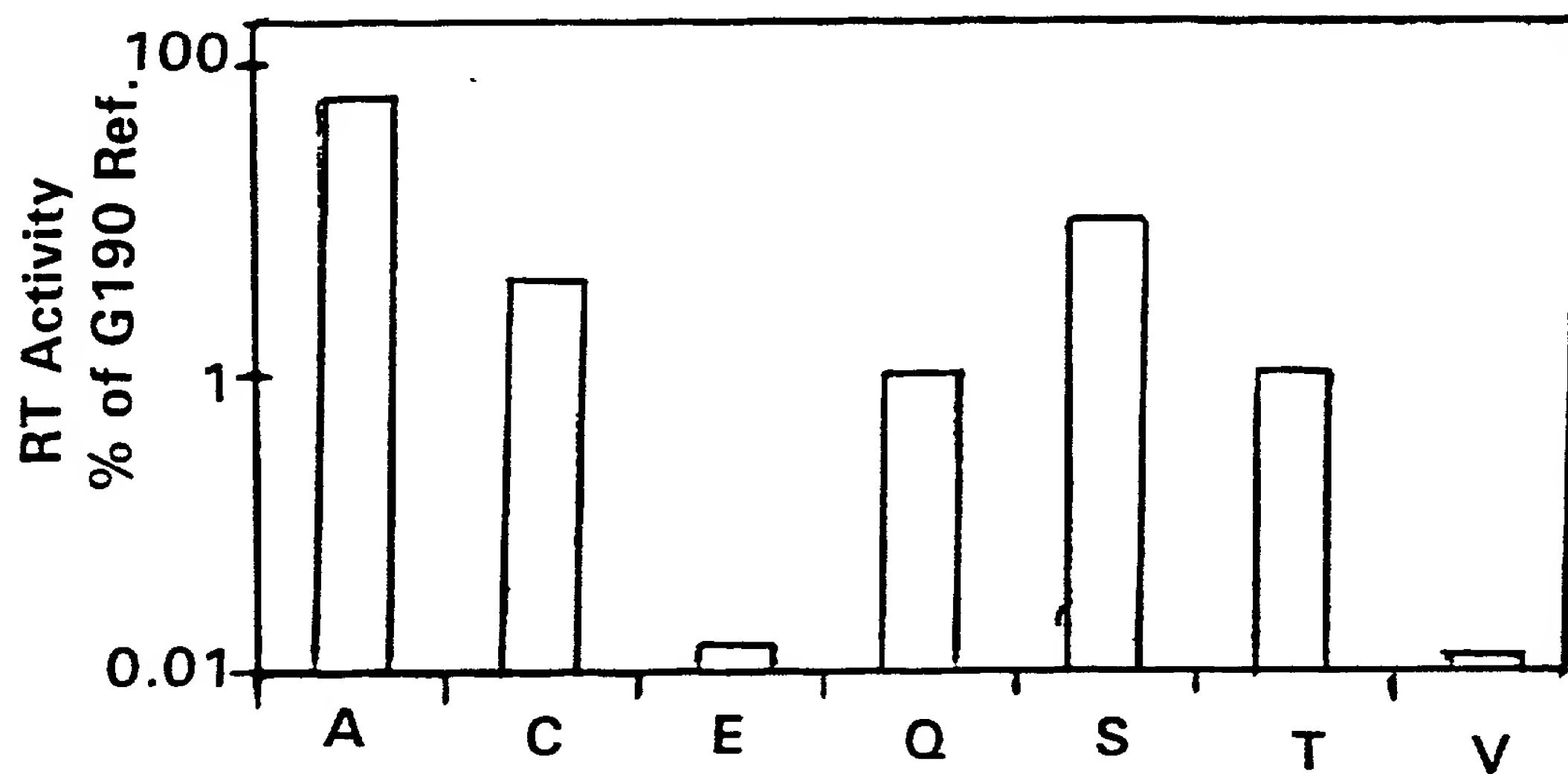
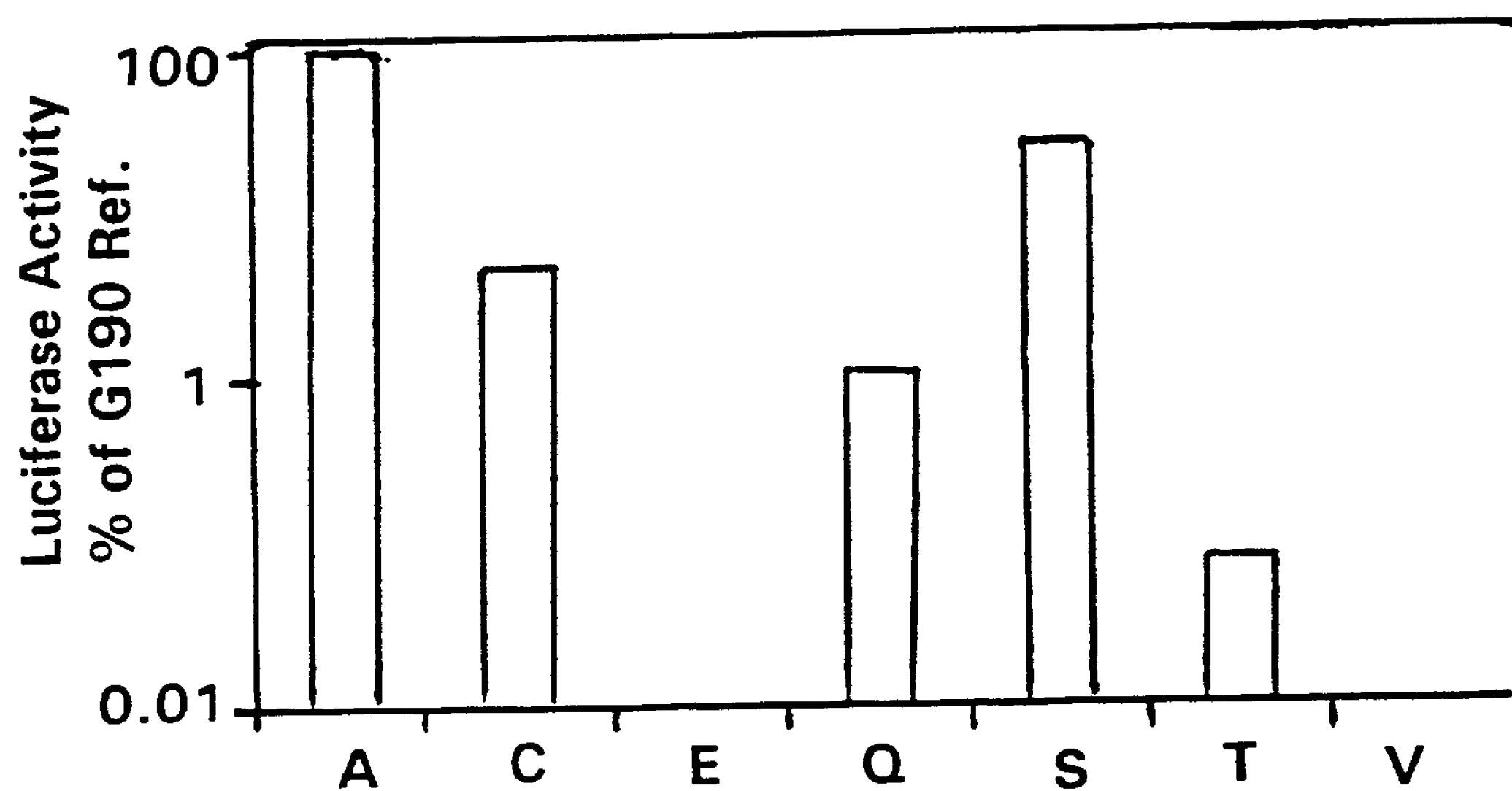


Figure C: Replication Fitness, PR Processing, and RT Activity



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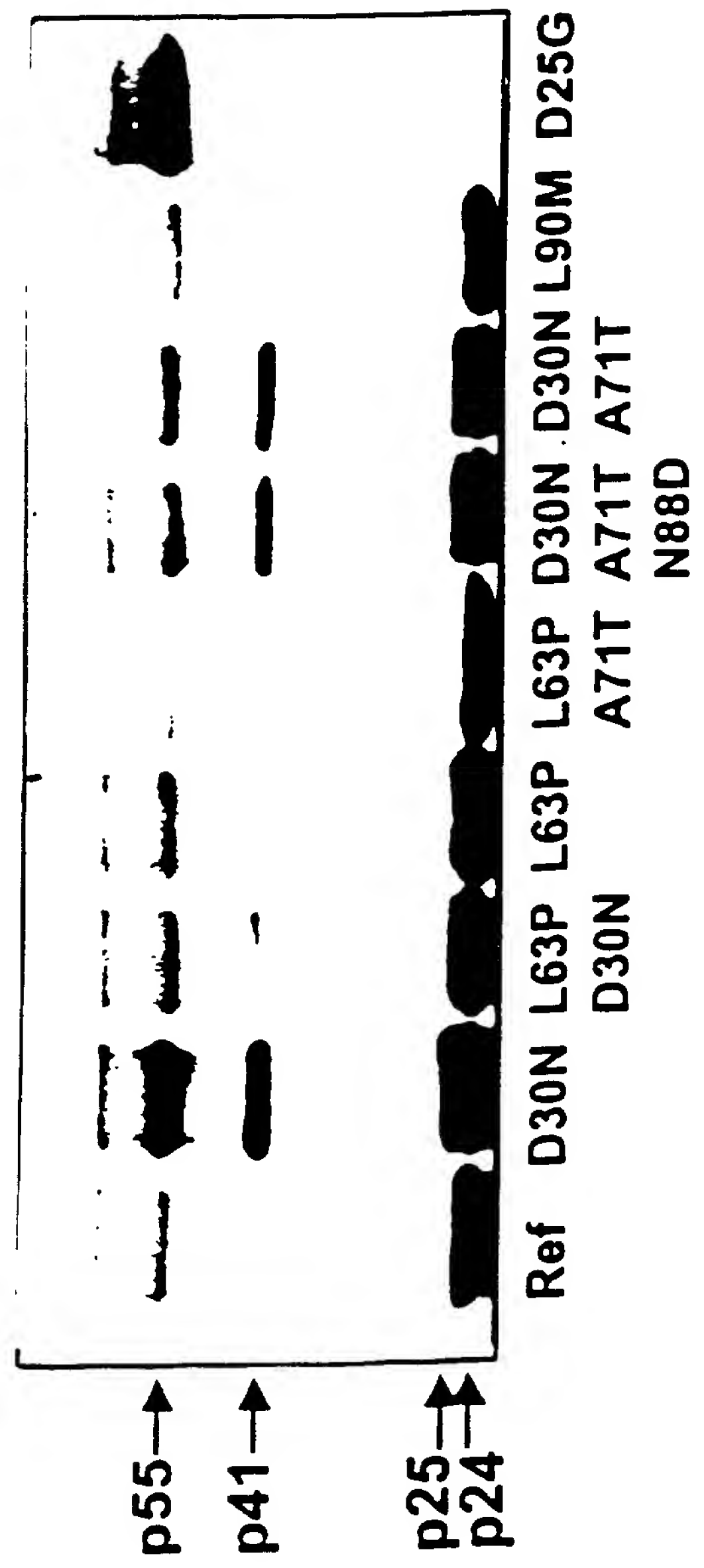
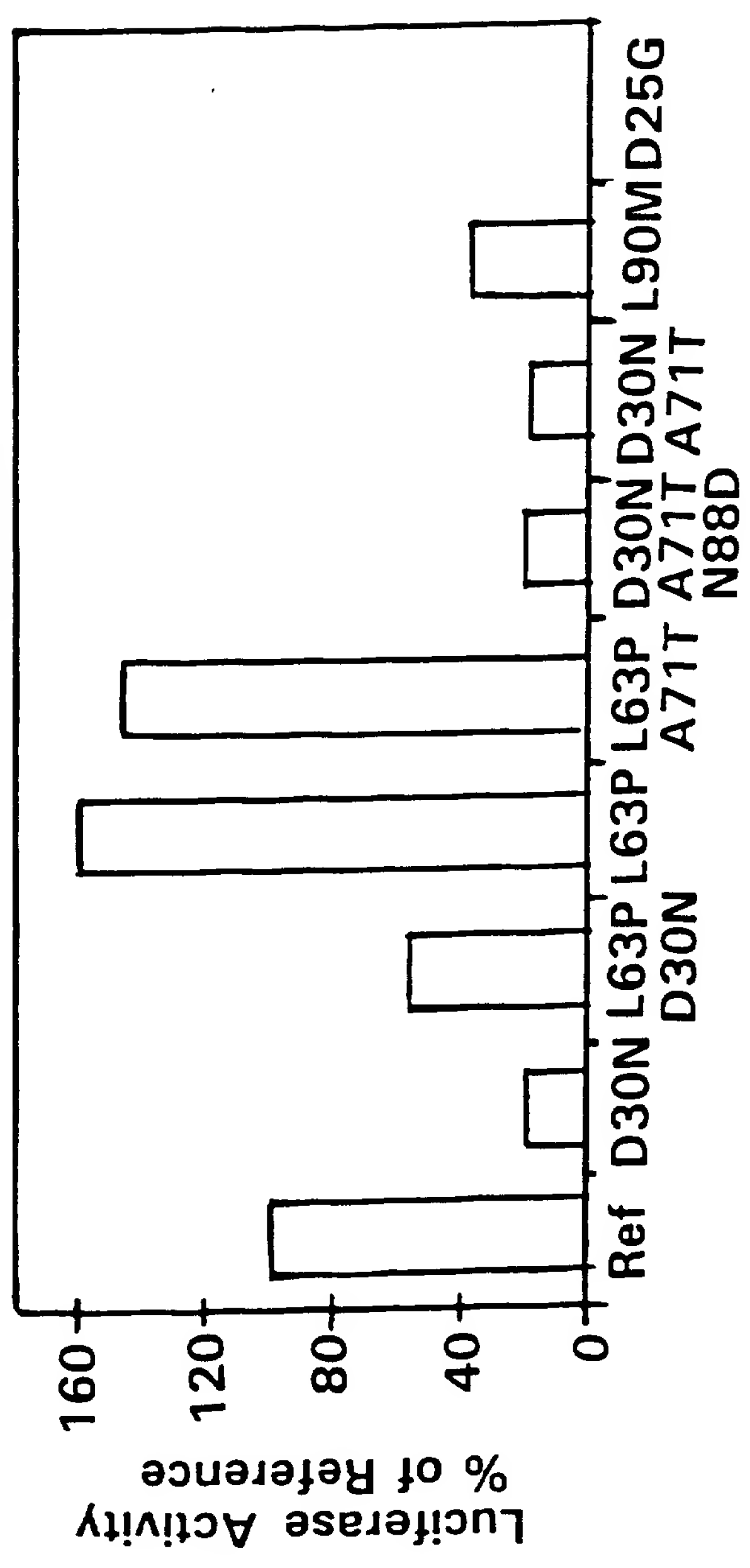
Figure D: Site Directed RT Mutants (G190 Series)



G190 Mutants

A = Ala	C = Cys
E = Glu	Q = Gln
S = Ser	T = Thr

Figure E: Site Directed PR Mutants



Replication Fitness and PR/RT Function

[illegible]

Replication Fitness and PR/RT Function

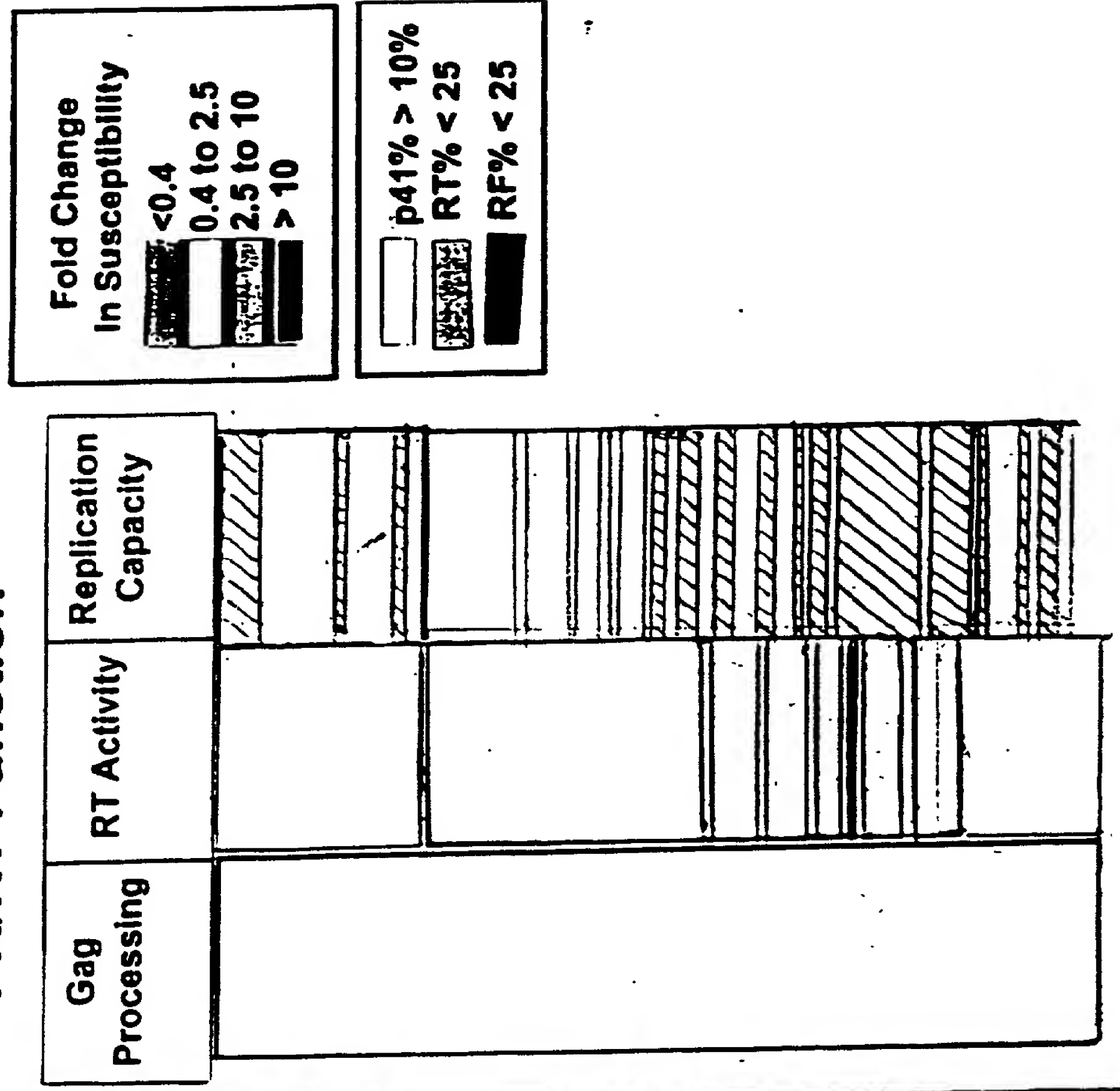


Figure G: Relation of PI Resistance to Replication Capacity

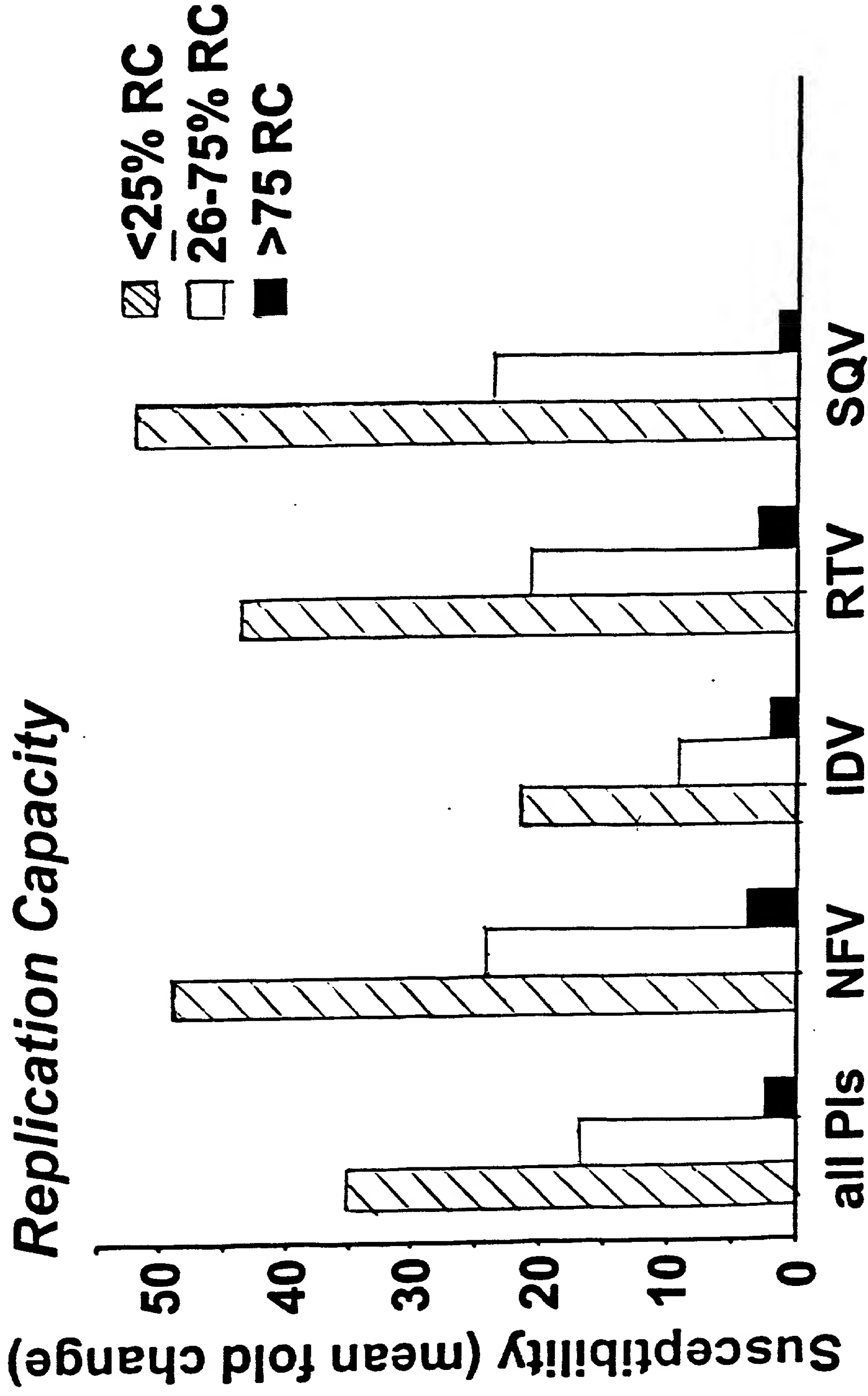


Figure H: Relation of NRTI and NNRTI Resistance to Replication Capacity

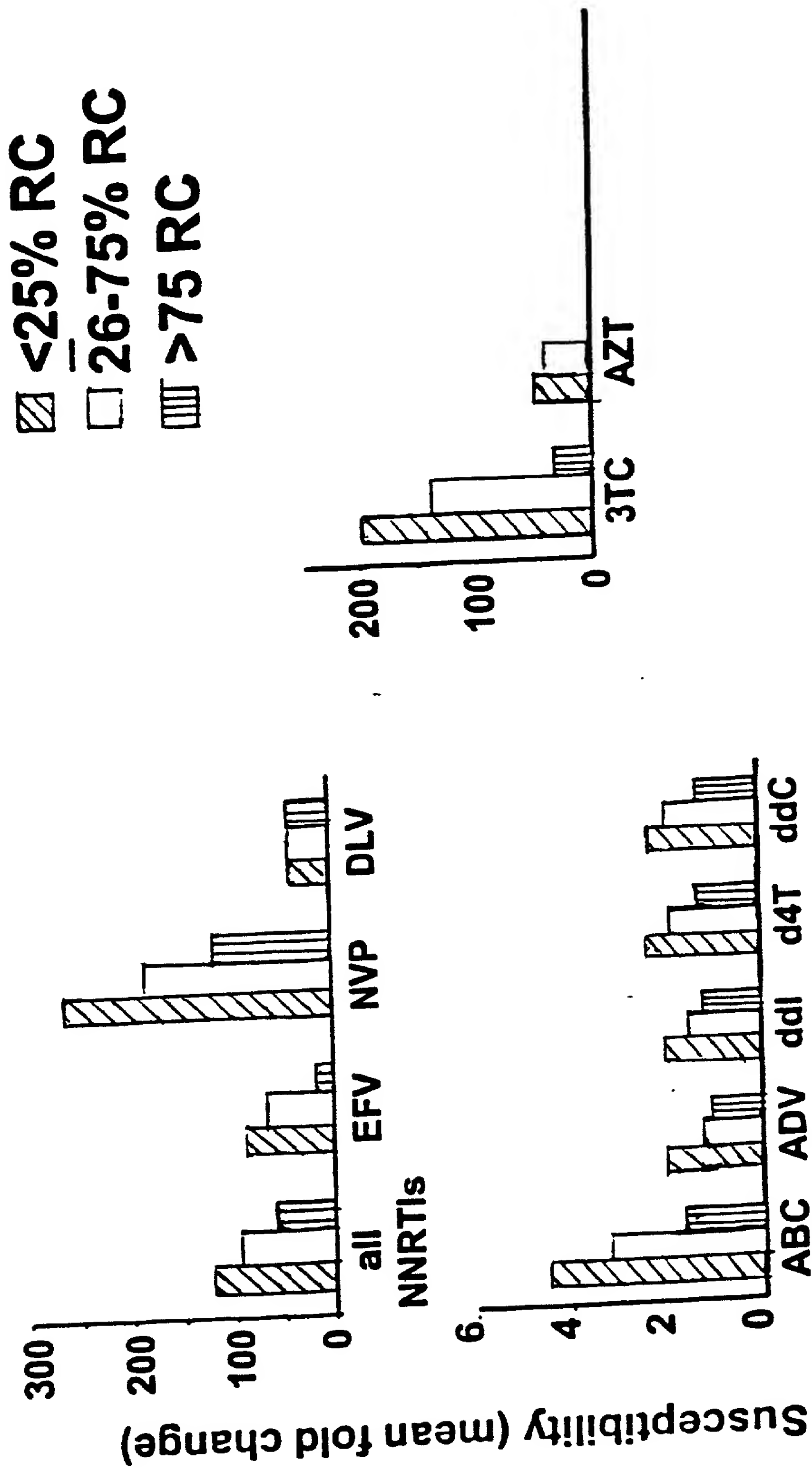


Figure I: Low Replication Capacity is Associated with High Numbers of Mutations in Protease and L90M

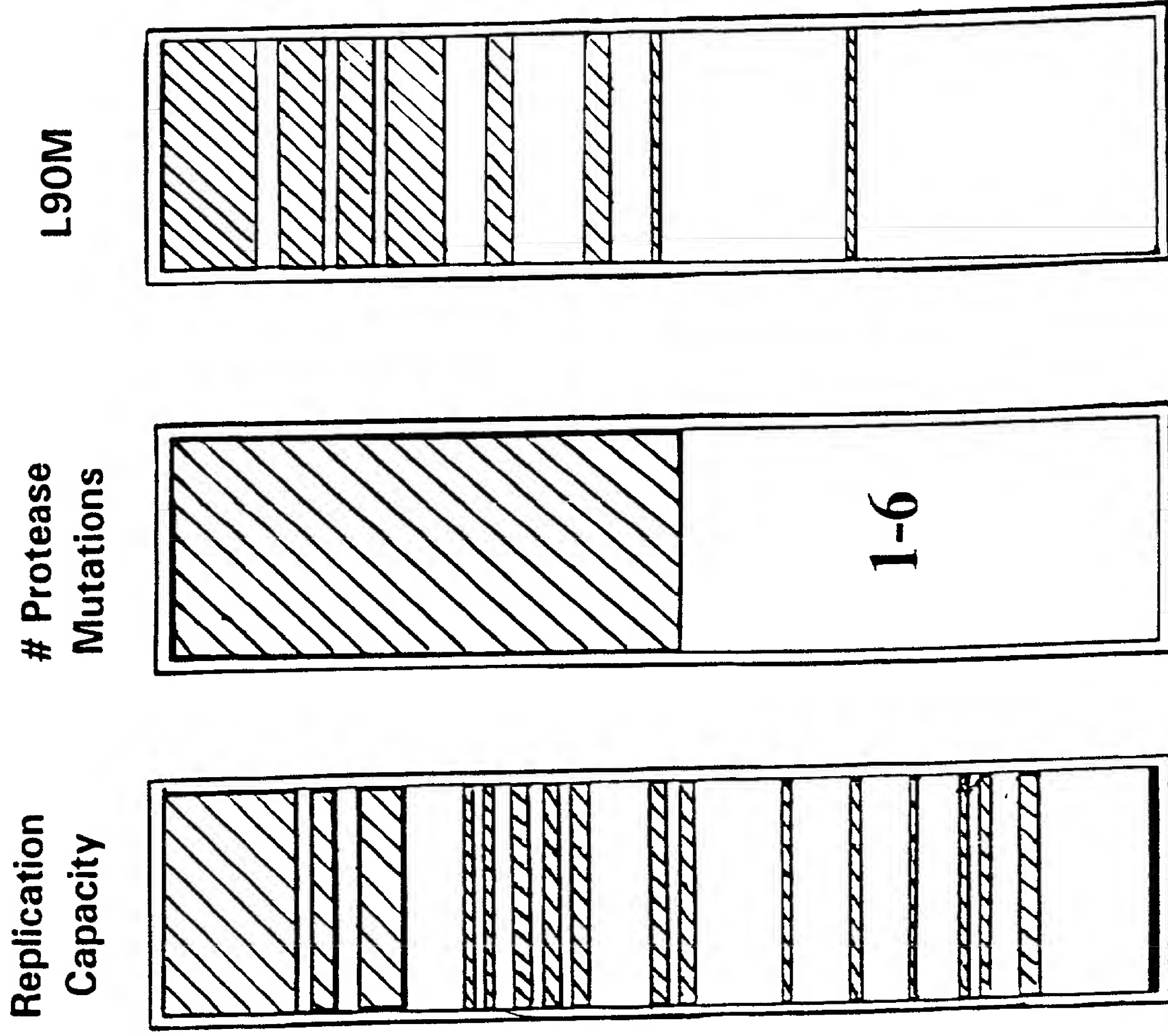
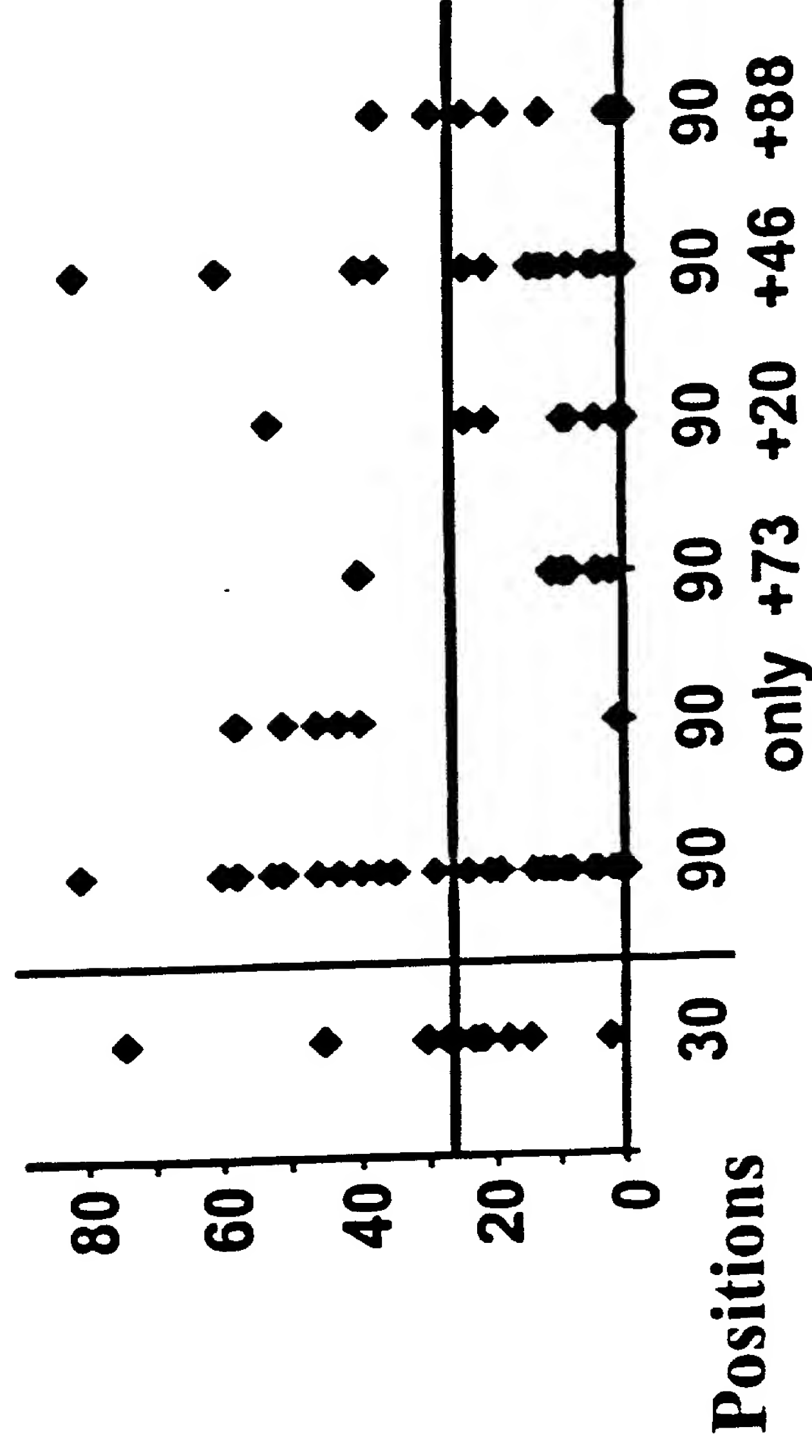


Figure J: Low Replication Capacity is Associated With Specific Protease Mutations

- **D30N**
- **L90M PLUS mutations at 73, 20, 46, or 88**



p value **.05**

$<.05$ $<.01$ $<.01$ $.06$

90.

Gag Processing

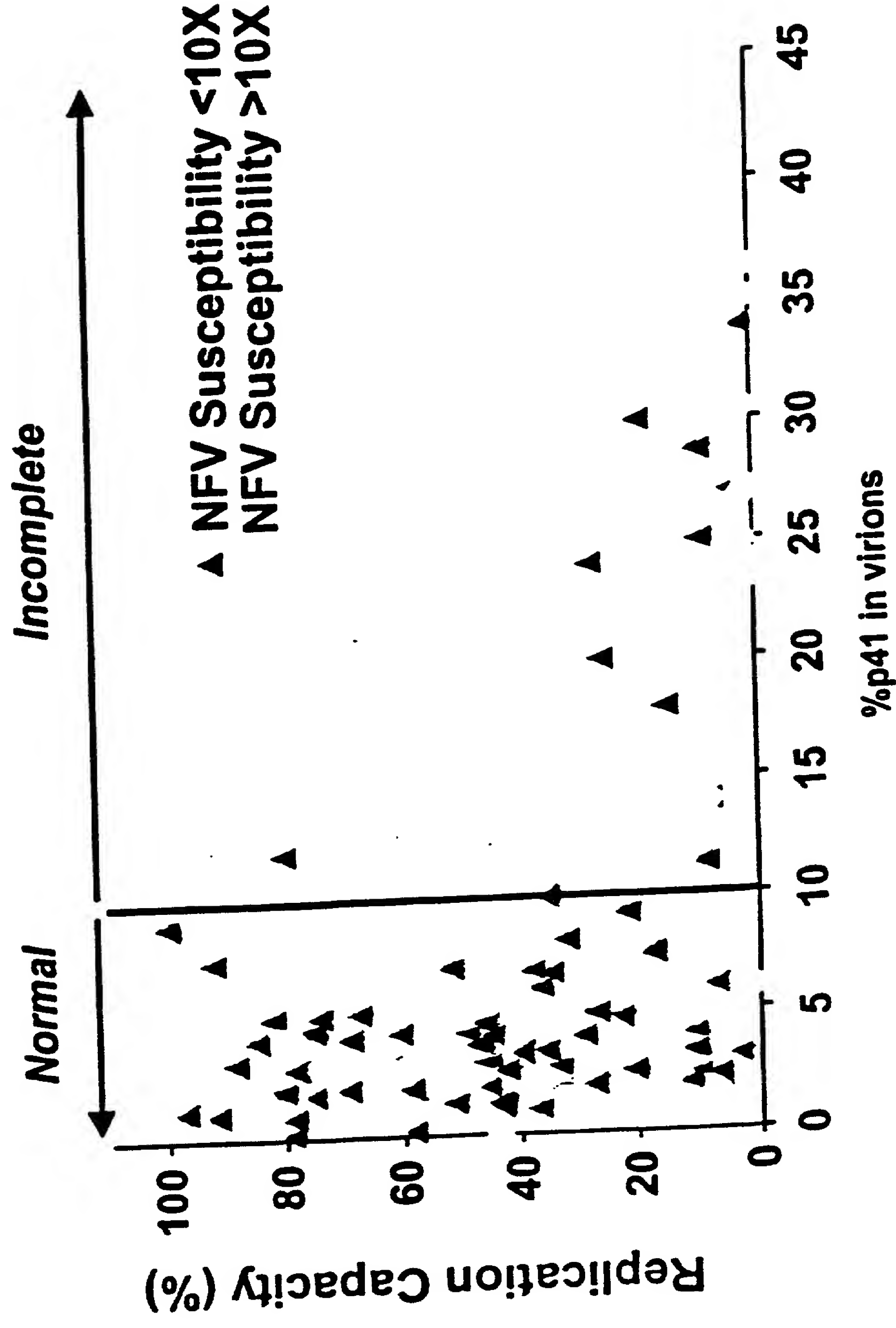
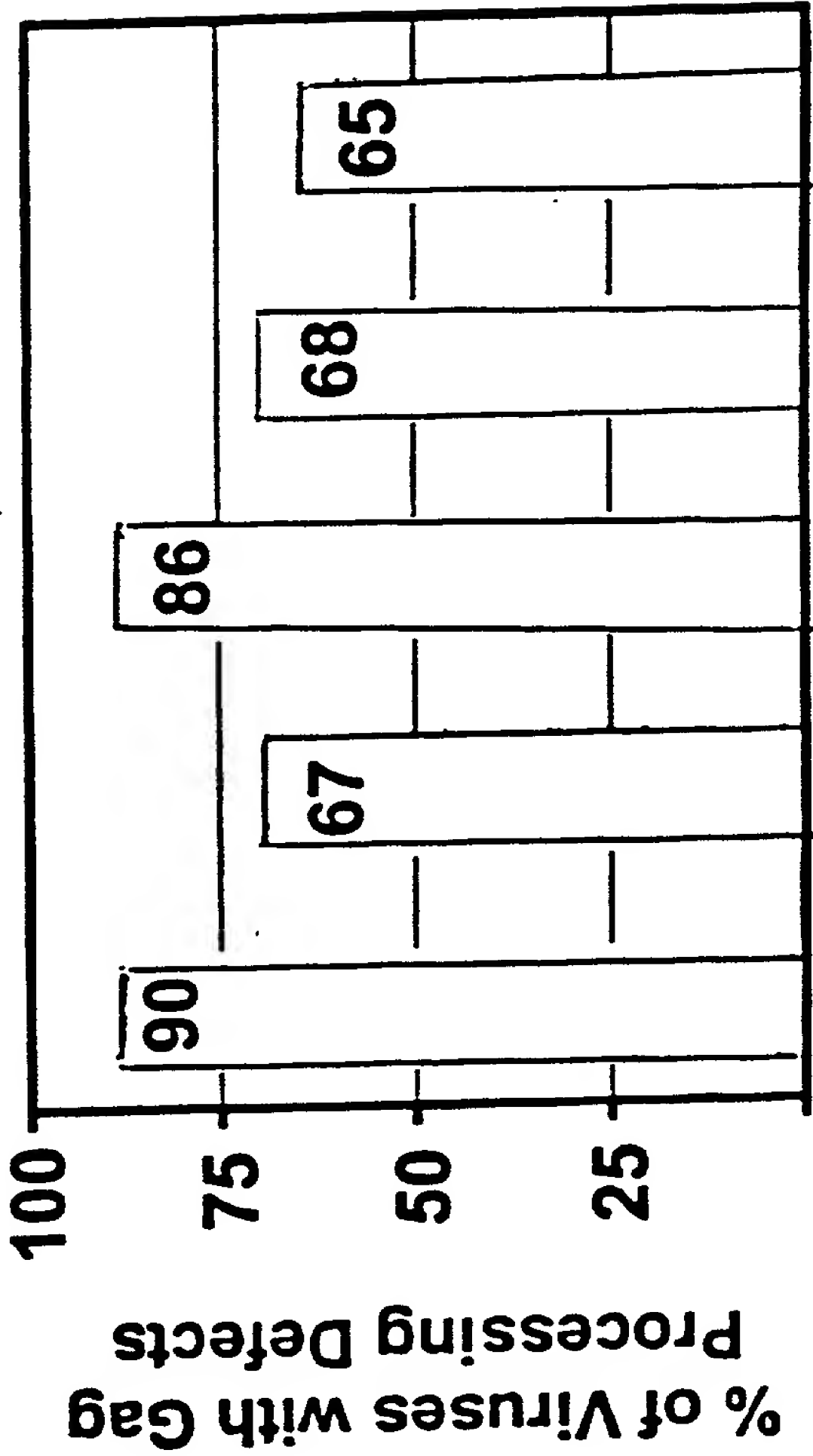


Figure L: Mutations in PR Associated with Gag Processing Defects

D30N **M46I/L** **G48V** **I54L/A/S/T/V** **I84V**



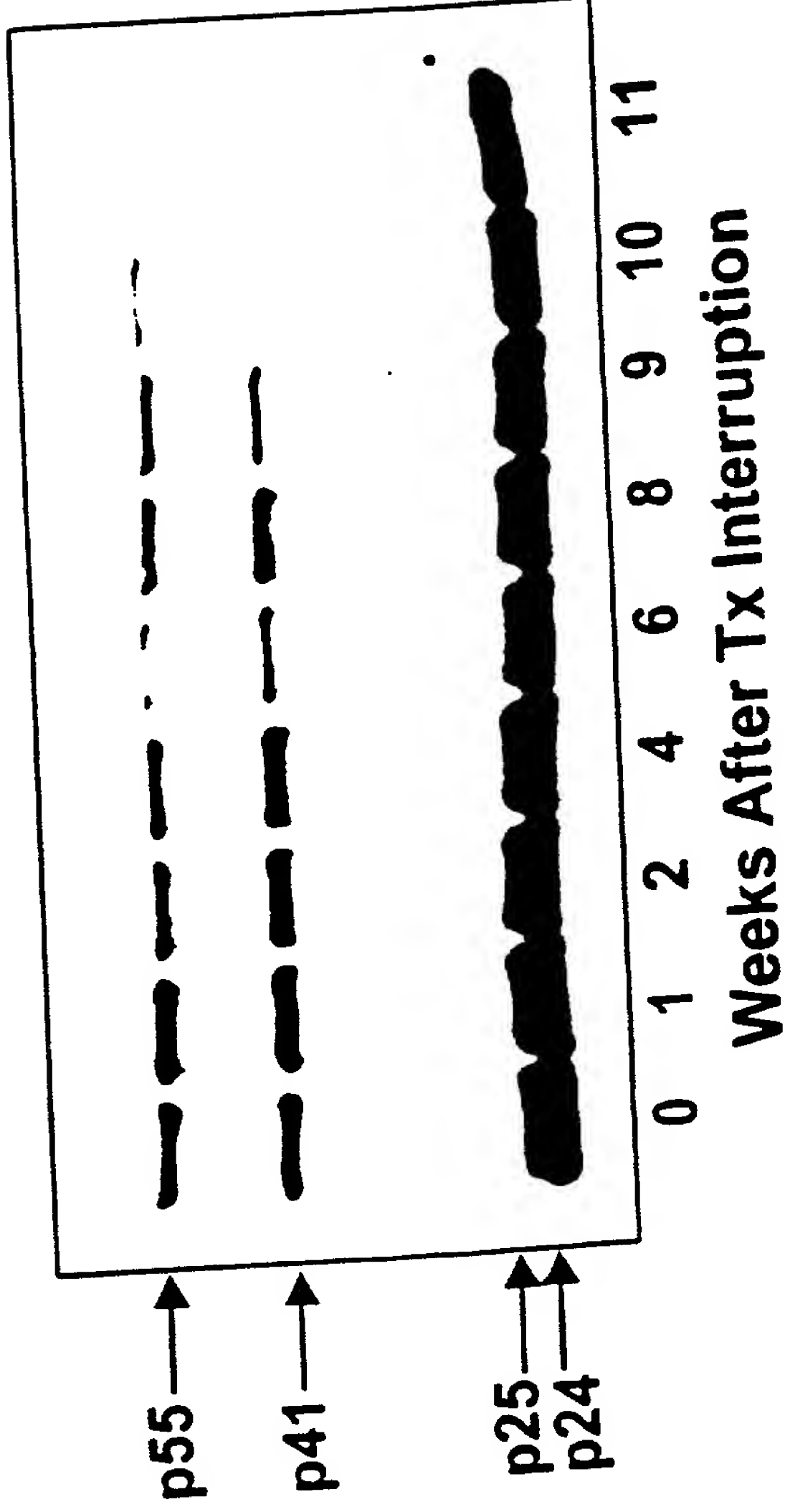
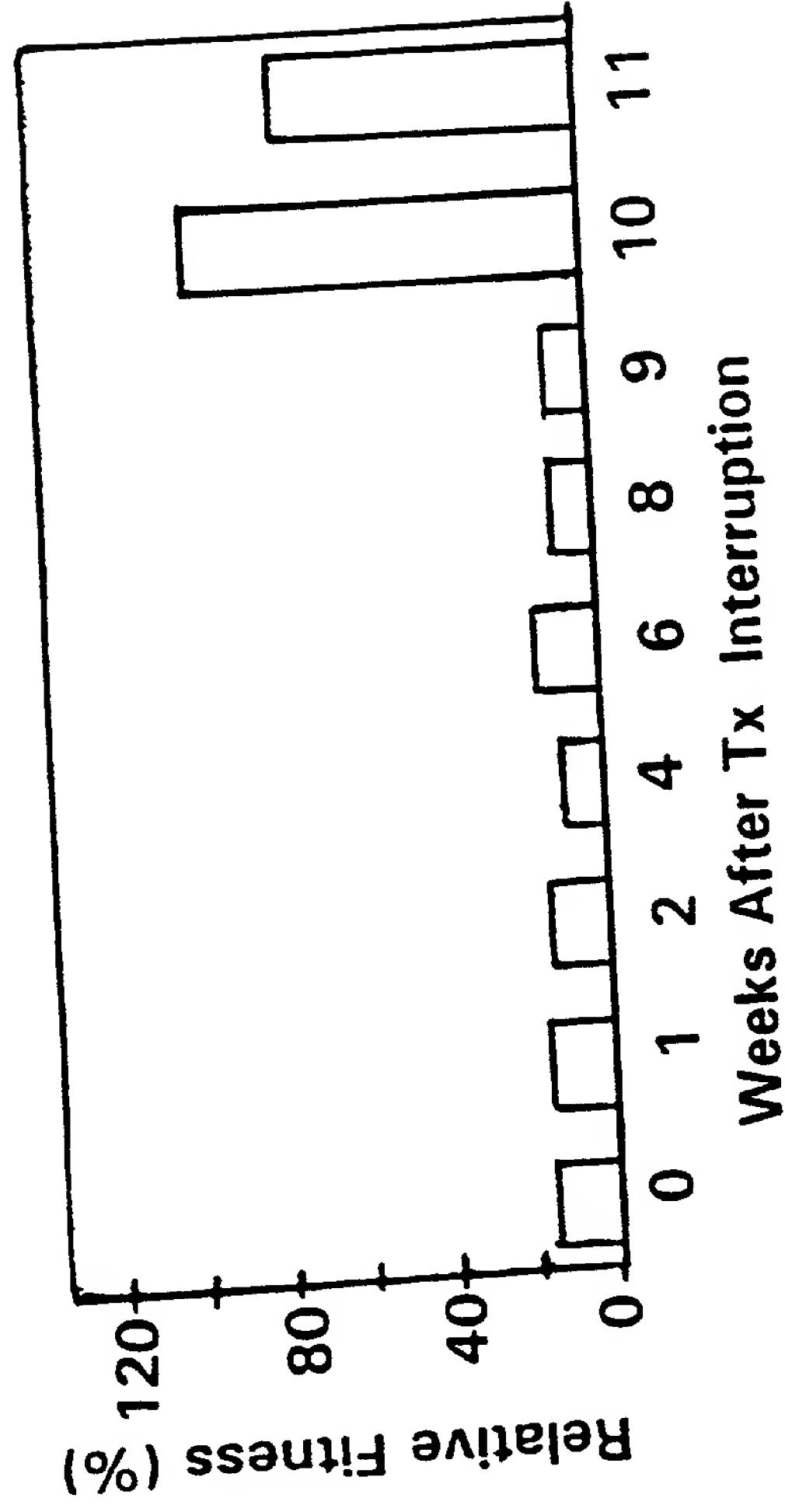
Position	30	46	48	54	84
p value	<0.1%	<0.1%	<1%	<0.1%	<1%
n	10	24	7	19	17

WEEK	NRTI				NNRTI				PI			
	AZT	3TC	D4T	ABC	NVP	DLV	EFV	SQV	IDV	RTV	NFV	AMP
day 0	3.7	>100	2.8	19	>300	88	115	85	72	73	74	16
1	4.5	>100	3.3	20	>300	78	134	95	74	59	80	21
2	5.8	>100	3.2	14	>300	75	142	89	77	49	59	19
3	6.5	>100	2.7	15	>300	96	183	59	75	52	51	15
4	6.3	>100	3.1	15	>300	94	174	59	68	50	49	15
5	6.4	>100	3.0	17	>300	76	119	59	60	54	36	10
6	5.0	>100	2.8	19	>300	93	168	89	39	80	40	18
7	9.1	>100	4.1	12	>300	89	154	85	78	53	53	19
9	2.8	8.1	1.9	5.0	22	15	10	1.8	3.5	4.7	4.0	2.0
10	1.5	1.7	1.1	1.3	1.7	2.0	1.6	0.9	1.6	1.9	1.8	1.6
11	0.9	1.2	1.0	1.2	0.8	1.1	0.9	1.0	1.1	1.1	1.1	1.0
12	0.8	1.3	0.8	1.2	0.5	1.0	0.8	0.8	0.8	0.9	1.1	0.8
23	0.7	1.1	1.0	0.6	0.8	1.1	0.8	0.8	0.8	1.0	0.9	0.6

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Figure M: Patient Virus Reversion to Drug Susceptibility after Treatment Interruption

Figure N: Patient Virus Reversion to Normal Replication Fitness after Treatment Interruption



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% of Control (CND0)

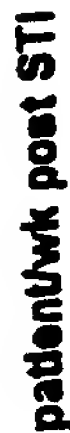


Figure P: **To Measure Replication Capacity of Patient-Derived Recombinant Viruses**

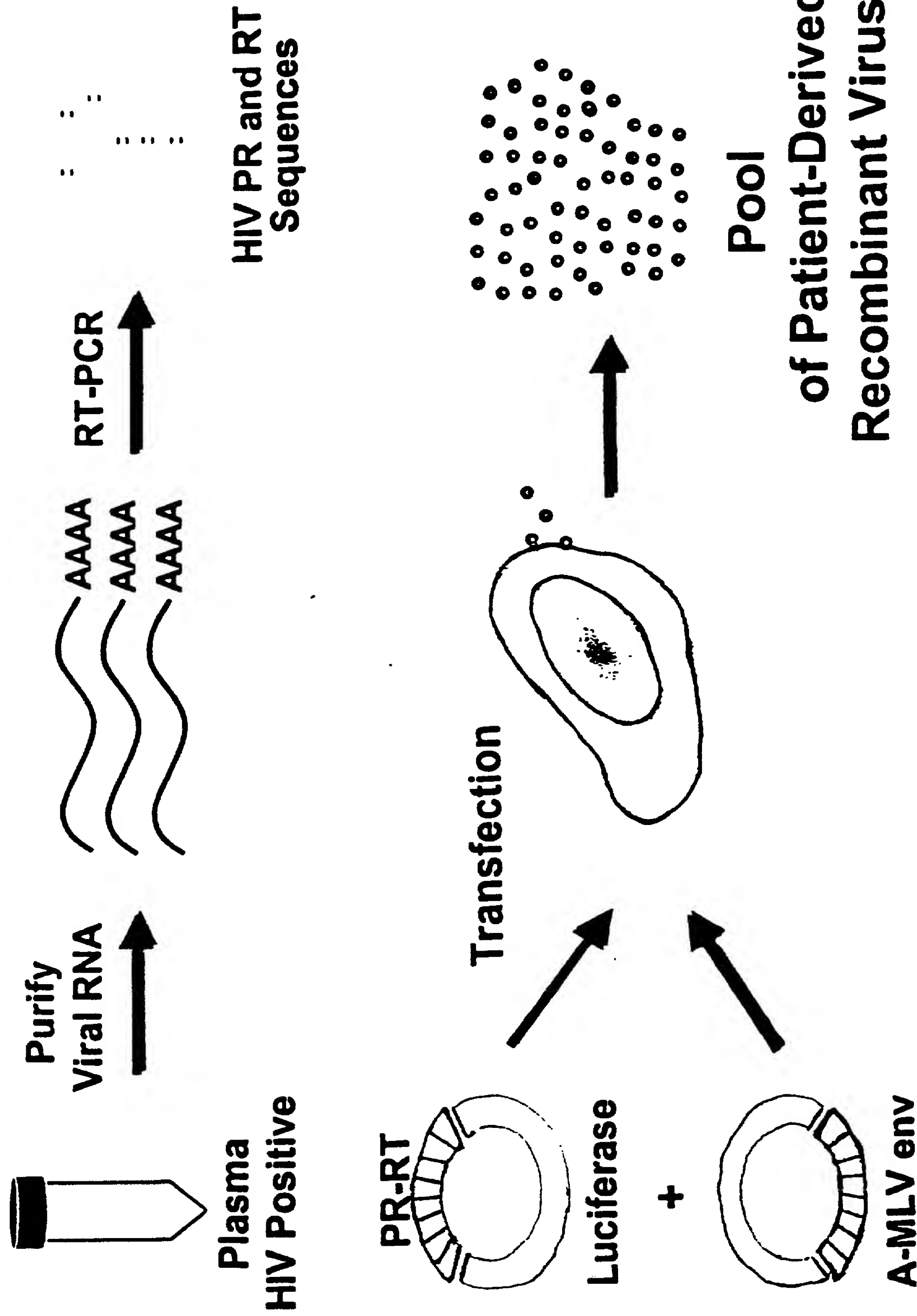
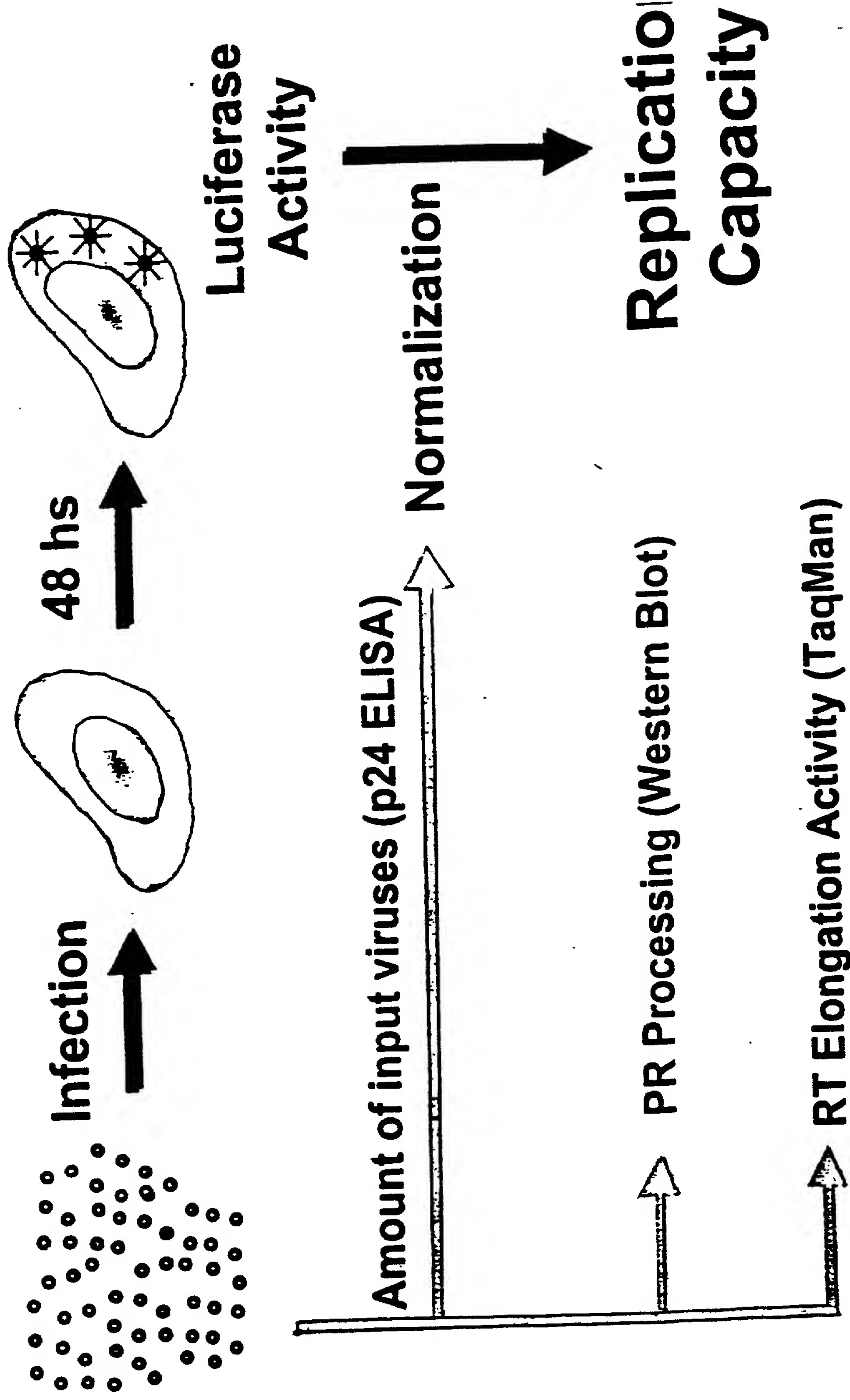
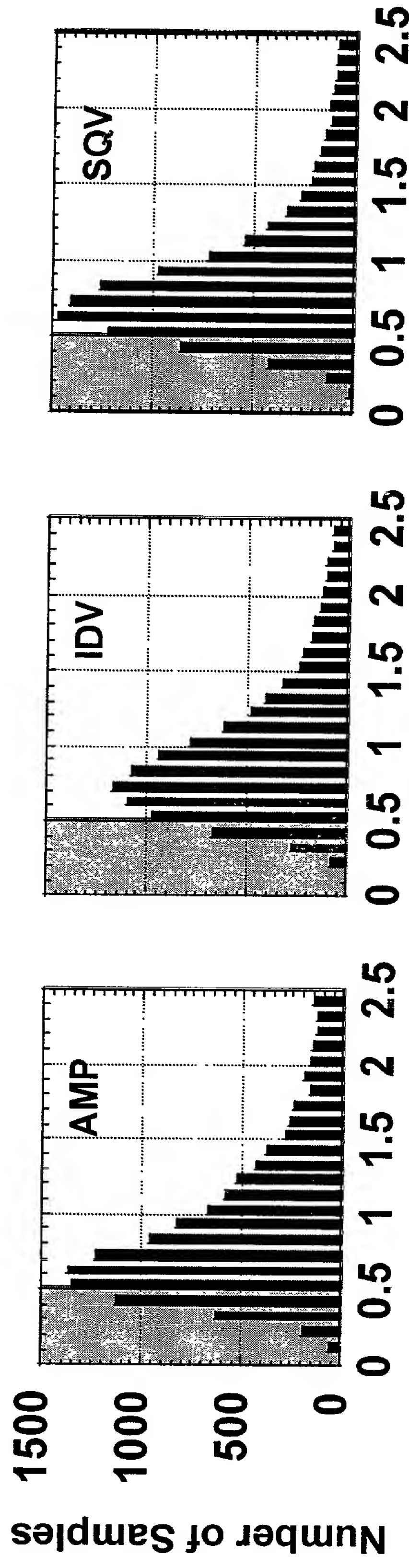


Figure Q:

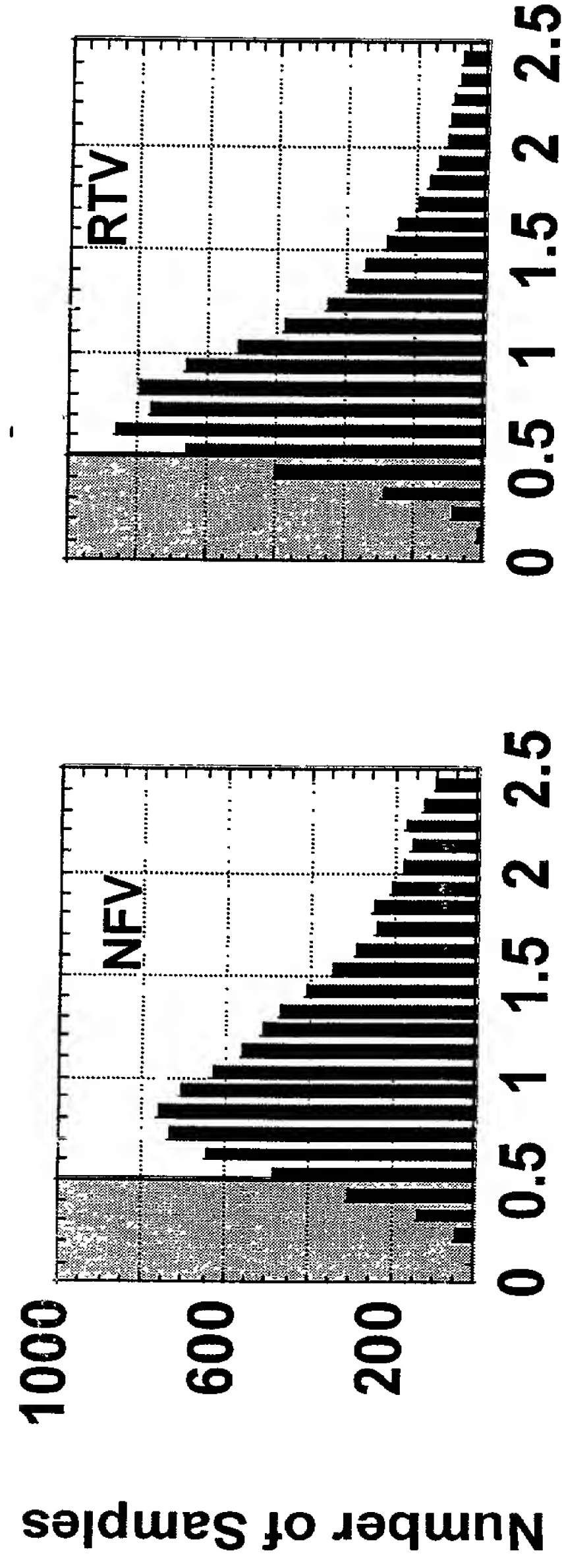
To Measure Replication Capacity of Patient-Derived Recombinant Viruses



Distribution of Fold Change in IC50s to



Fold Change in IC50 with Respect to the Reference



Fold Change in IC50 with Respect to the Reference

Fold Change Susceptibility

20 Randomly Selected Patient Derived Viruses with HS to PIs

RT Inhibitors

PR Inhibitors

Sample	ABC	ddI	3TC	d4T	ddC	ZDV	DLV	EFV	NVP	AMP	IDV	NFV	RTV	SQV
1	3.2	2.2	>300	0.9	1.7	1.2	0.9	41.9	>700	0.4	0.6	1.1	0.4	0.3
2	1.0	1.0	1.3	1.1	1.1	0.7	1.2	0.8	0.8	0.6	0.3	0.7	0.2	0.3
3	3.1	1.7	>300	0.9	nd	0.7	nd	1.1	0.8	0.2	0.4	0.6	0.4	0.3
4	3.3	1.9	>300	1.0	2.4	1.2	62.9	101	429	0.2	0.4	0.6	0.4	0.2
5	3.6	2.2	50	1.7	3.2	0.6	>190	>320	>700	0.2	0.4	0.6	0.5	0.3
6	7.5	1.4	>300	1.4	2.1	22.9	12.8	135	>700	0.5	0.5	0.6	0.4	0.4
7	8.5	1.9	>300	3.7	3.4	73.9	30.6	>320	>700	0.3	0.4	0.6	0.3	0.4
8	2.7	1.6	>300	1.0	1.8	1.1	>190	89.3	>700	0.4	0.4	0.5	0.6	0.4
9	2.0	1.1	>300	0.7	1.3	0.8	130	72.1	165	0.3	0.4	0.5	0.3	0.5
10	2.4	1.7	>300	1.2	1.9	0.6	71.5	38.7	109	0.4	0.4	0.4	0.4	0.4
11	2.8	1.5	>300	0.7	1.7	0.4	30.9	94.9	193	0.4	0.4	0.4	0.5	0.4
12	3.4	1.1	>300	1.0	2.1	0.7	3.2	2.0	2.6	0.3	0.5	0.4	0.5	0.4
13	3.1	2.1	>300	1.1	3.8	0.6	2.4	1.1	1.5	0.3	0.3	0.4	0.3	0.3
14	1.6	1.1	2.0	0.9	1.5	0.9	>190	60.4	>700	0.2	0.3	0.3	0.2	0.2
15	1.2	1.0	1.2	1.1	1.2	1.7	1.2	1.2	1.2	0.2	0.4	0.3	0.4	0.6
16	2.8	1.3	3.5	1.2	1.2	14.3	21.9	12.4	71.8	0.2	0.3	0.2	0.2	0.4
17	3.0	2.0	>300	1.2	1.8	2.0	11.3	22.1	160	0.2	0.2	0.2	0.2	0.2
18	3.9	1.4	>300	1.6	1.5	3.1	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.3
19	3.1	1.1	49.5	1.6	1.5	6.9	13.4	9.9	33.2	0.3	0.2	0.2	0.2	0.2
20	0.9	1.2	1.3	0.9	0.8	1.0	0.8	0.6	0.6	0.3	0.3	0.2	0.3	0.3

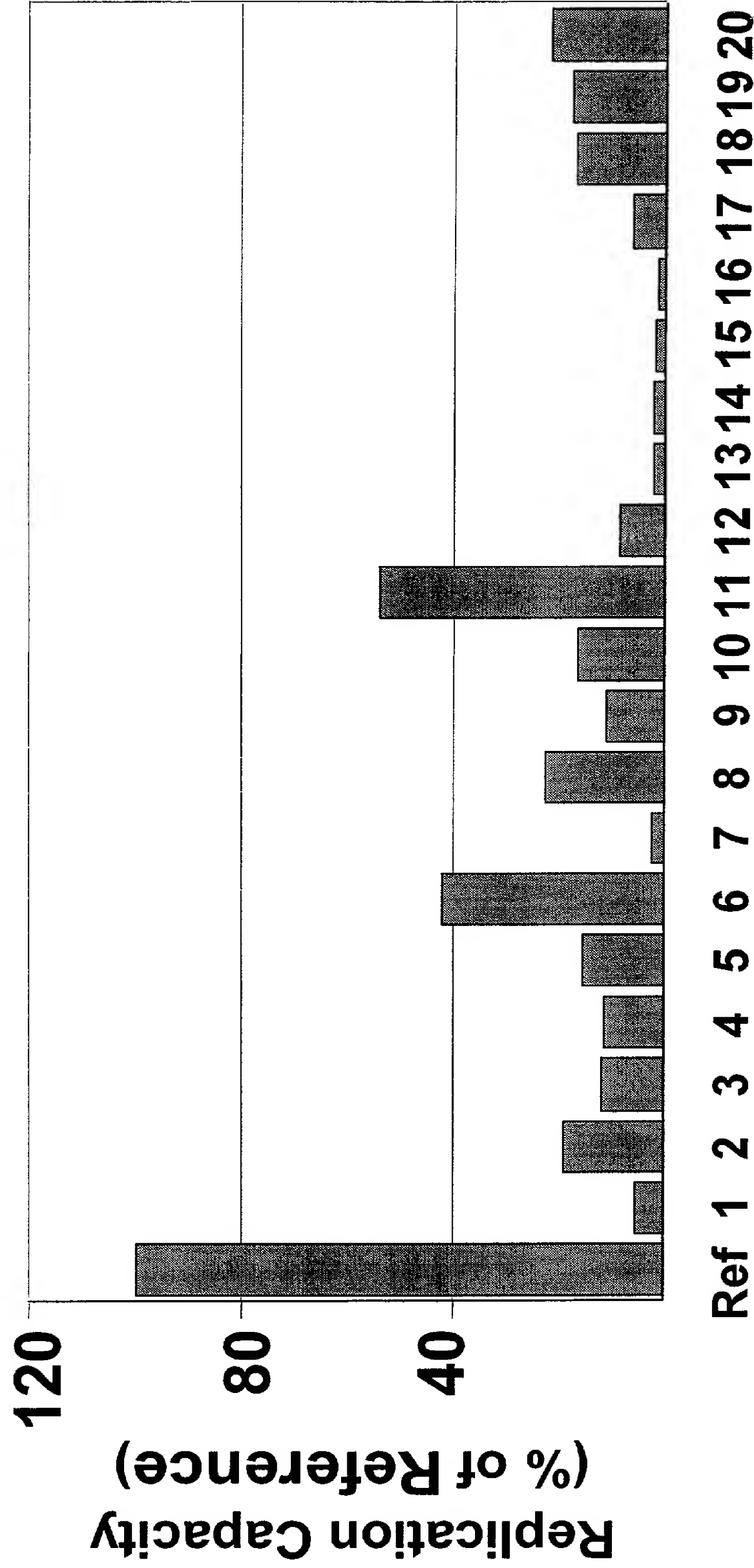
0 - 0.4

0.4 - 2.5

2.5 - 10

> 10

Replication Capacity of Patient Derived Viruses with HS to Pls



Patient Derived Viruses

Figure 9
F01090-2474350

PhenoSense™ HIV

Cell based assay to measure phenotypic drug susceptibility
employing patient-derived recombinant viruses

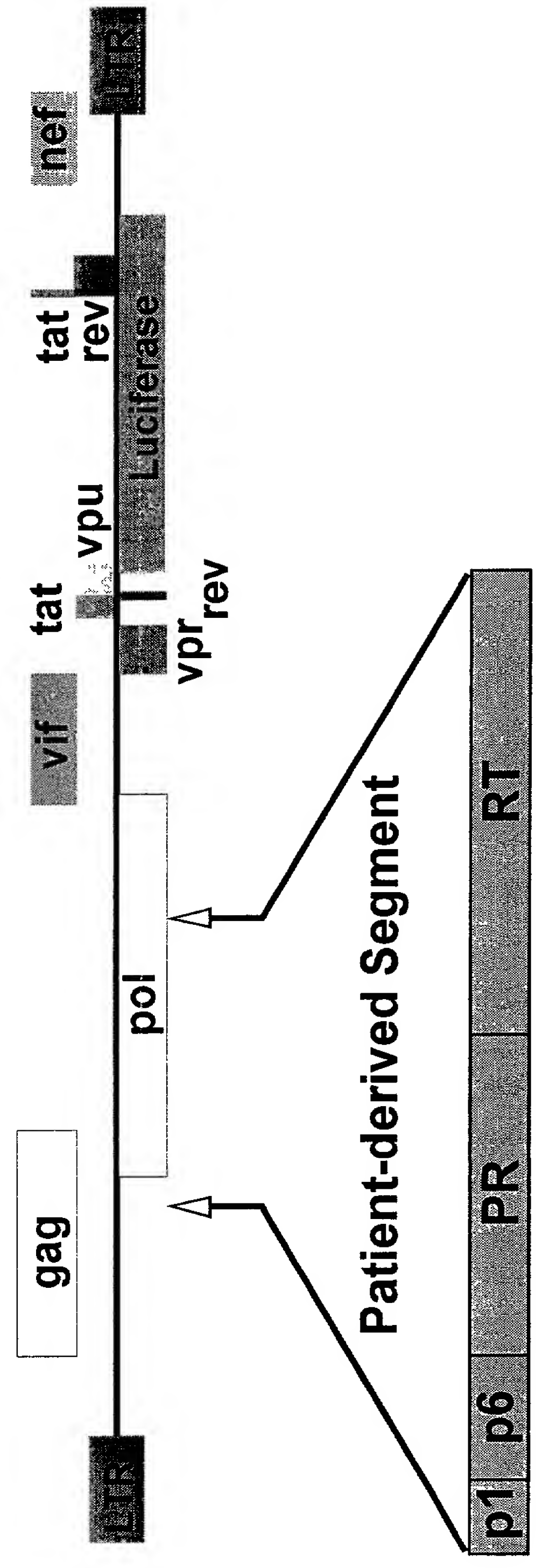


Figure 10
PhenoSense

In order to identify mutations responsible for HS and decreased fitness, we used a modified PhenoSense HIV assay employing recombinant viruses carrying different segments from patient isolates:

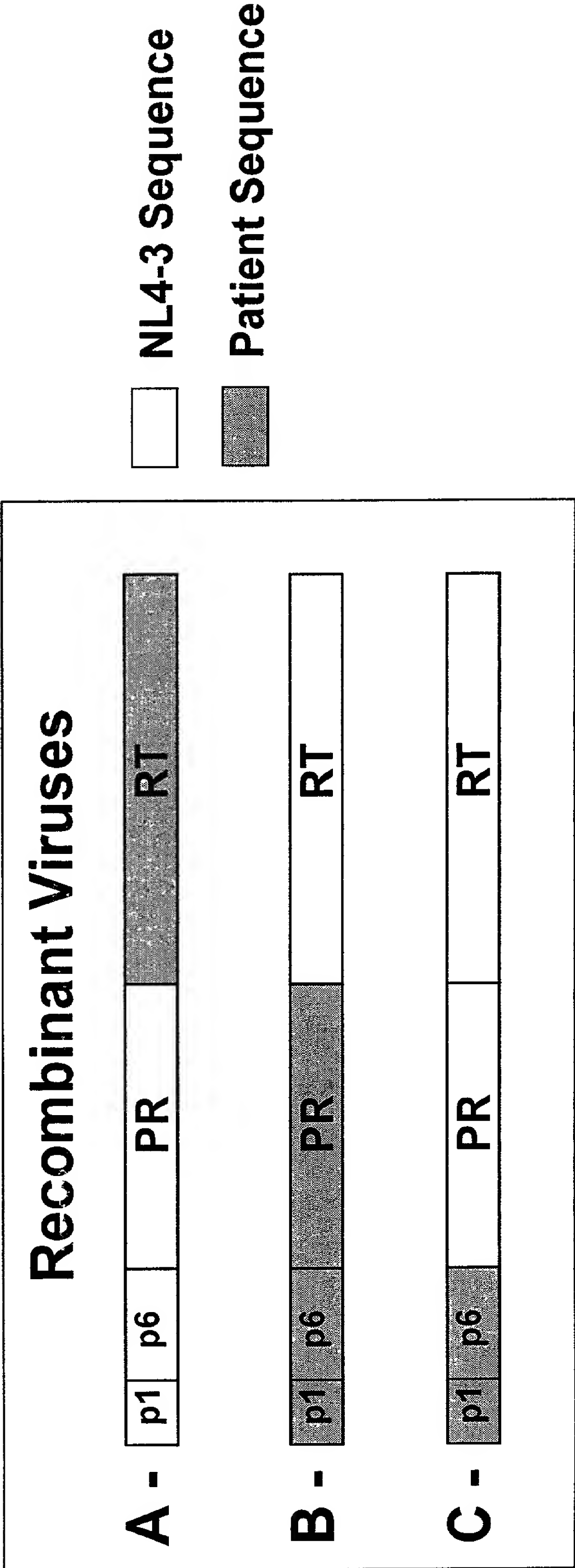


Figure 11

A -

NL4-3 Sequence

Patient Sequence

Fold Change in Susceptibility

Sample	ABC	ddl	3TC	d4T	ddC	ZDV	DLV	EFV	NVP	AMP	IDV	NFV	RTV	SQV
1	2.1	1.5	>300	0.8	1.5	0.8	0.7	35.8	>700	0.7	1.0	1.1	0.9	0.9
2	1.0	1.2	1.4	1.0	1.1	0.7	1.5	0.8	0.8	0.7	0.8	1.0	0.9	0.8
3	4.5	1.8	>300	0.9	2.1	0.7	2.1	1.1	1.4	0.6	0.9	0.9	0.7	0.4
4	3.5	1.8	>300	0.9	1.8	1.1	85.9	141	344	0.6	0.8	0.9	0.8	0.8
5	2.7	2.1	>300	1.4	2.1	0.5	>190	>320	>700	0.5	1.0	1.1	0.7	1.0
6	7.0	1.4	>300	1.5	2.6	0.8	39.3	189	>700	0.7	0.5	0.8	0.7	0.7
7	9.9	2.6	>300	1.3	2.9	80.1	48.1	>320	>700	0.7	0.8	0.9	0.8	0.5
8														
9	1.9	1.1	>300	1.2	1.1	1.1	31.4	170	>700	0.7	0.7	1.4	0.8	0.9
10	3.8	1.8	>300	0.9	2.3	0.8	73.3	50	100	0.7	0.8	1.0	0.8	1.0
11	2.3	1.5	>300	0.7	1.7	0.5	35.6	130	182	0.6	1.1	1.0	1.0	0.8
12	4.3	1.9	>300	0.9	2.3	0.8	2.2	1.2	1.5	0.9	0.9	1.2	1.0	1.0
13	3.4	1.6	>300	1.0	2.1	0.4	2.1	0.8	1.2	0.8	1.0	1.0	1.0	1.0
14	5.7	1.8	>300	1.8	2.2		0.5	0.6	0.7	0.5	0.5	0.7	0.8	0.7
15	1.6	1.1	1.0	1.0	1.0	1.6	1.1	1.2	1.2	0.8	1.1	1.2	1.0	1.1
16	3.3	1.3	>300	1.4	1.3	31	47.9	25	106	0.5	0.5	0.8	0.6	0.7
17	3.9	1.6	>300	0.8	2.0	2.2	12.6	33	166	0.5	0.8	0.7	0.9	0.7
18	5.7	1.8	>300	1.8	2.2		0.5	0.6	0.7	0.5	0.5	0.7	0.8	0.7
19	4.4	1.6	79.1	1.3	1.8	20	29	24	78	0.3	0.6	0.6	0.5	0.7
20	1.0	1.1	1.0	1.1	1.1	0.8	1.1	0.6	0.6	1.0	1.1	1.2	1.1	1.2

0 - 0.4

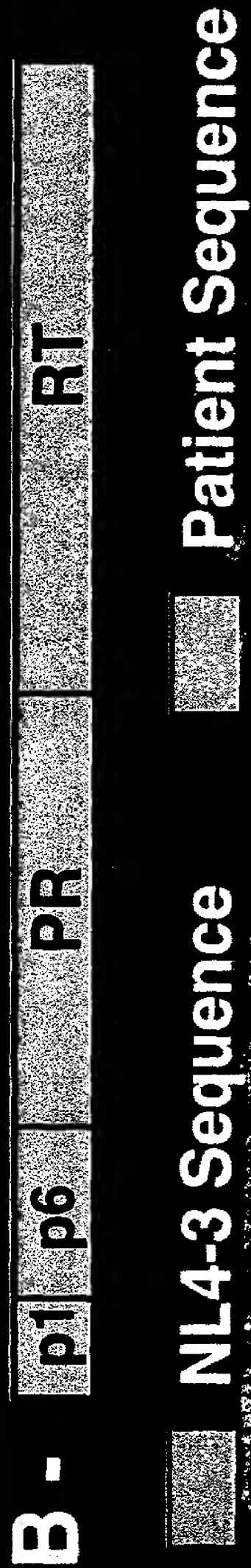
0.4 - 2.5

2.5 - 10

> 10

Figure 12

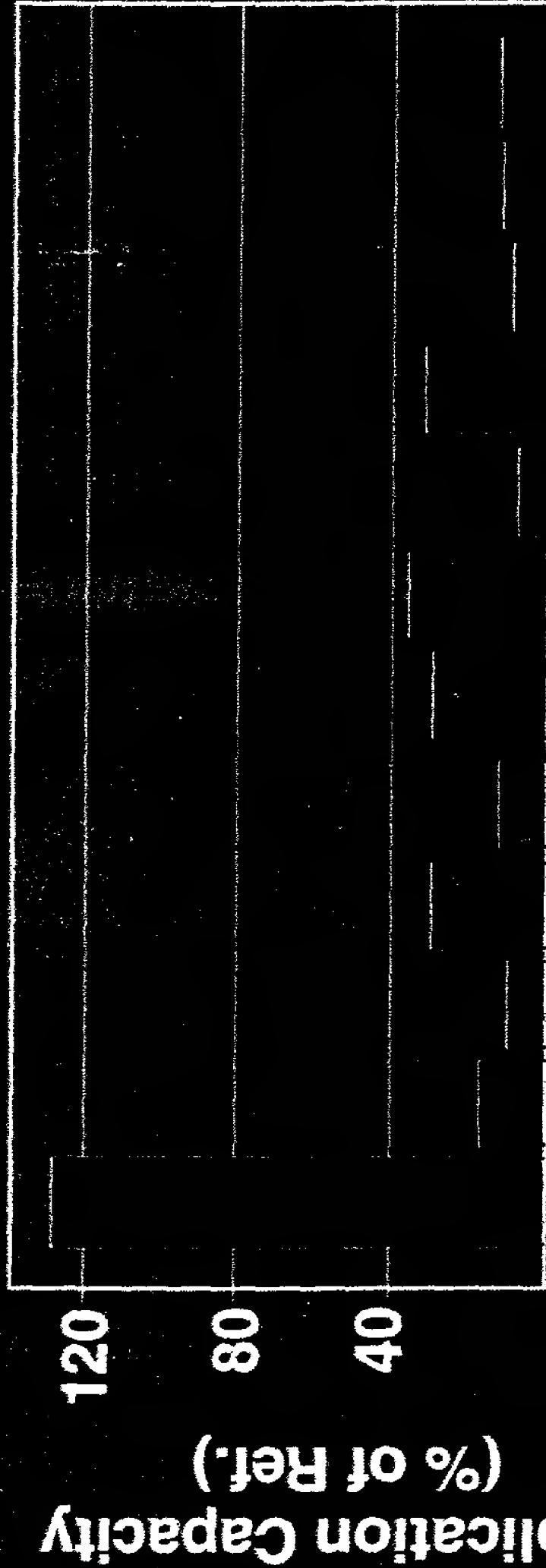
Table 24b



Fold Change in Susceptibility

Sample	ABC	ddl	3TC	d4T	ddC	AZT	DLV	EFV	NVP	AMP	IDV	NFV	RTV	SQV
1	0.9	0.9	1.0	1.0	0.9	0.8	0.7	0.8	0.8	0.4	0.6	1.3	0.7	0.5
2	1.0	1.0	1.0	0.9	1.1	1.1	0.6	0.7	0.7	0.6	0.3	0.6	0.2	0.2
3	0.8	1.0	1.0	1.0	0.9	0.9	0.6	0.7	0.6	0.3	0.7	0.7	0.4	0.5
4	0.9	0.9	0.7	1.2	0.9	0.9	0.7	0.8	0.9	0.3	0.5	0.7	0.4	0.4
14	0.9	1.0	1.0	0.9	0.9	0.7	0.7	0.9	0.5	0.3	0.5	0.6	0.7	0.9
15	0.9	1.1	0.9	1.1	1.0	1.1	0.9	0.9	0.7	0.2	0.3	0.3	0.3	0.6
16	0.8	1.0	0.8	1.1	1.1	0.7	0.5	0.8	0.7	0.4	0.3	0.3	0.4	0.5
17	1.0	1.0	0.9	1.0	1.0	1.0	0.7	1.0	0.8	0.2	0.4	0.5	0.4	0.6
18	0.9	0.7	0.8	0.9	0.9	0.9	0.6	0.9	0.5	0.3	0.4	0.4	0.4	0.5
19	0.9	1.0	0.9	0.8	1.0	0.8	0.7	0.9	0.8	0.4	0.4	0.4	0.3	0.6
20	0.9	1.0	1.0	0.9	0.9	1.0	0.6	0.9	0.6	0.2	0.3	0.3	0.3	0.4

Replication Capacity



Ref 1 2 3 4 14 15 16 17 18 19 20

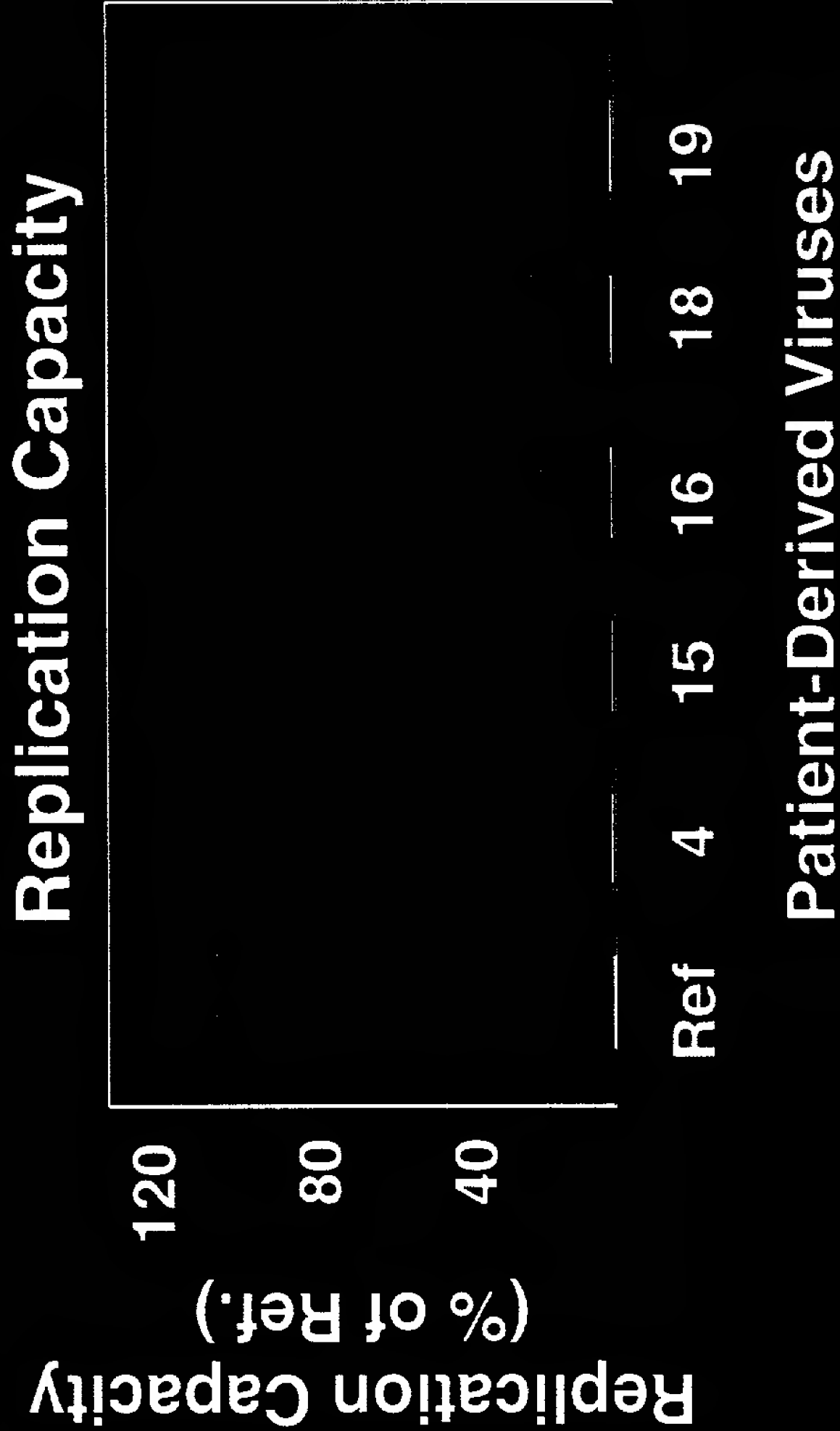
Patient-Derived Viruses

Figure 13



Fold Change in Susceptibility

Sample	ABC	ddl	3TC	d4T	ddC	ZDV	DLV	EFV	NVP	AMP	IDV	NFV	RTV	SQV
4	0.9	1.0	0.9	0.9	0.7	0.8	1.1	0.7	0.6	0.6	0.5	0.6	0.6	0.4
15	0.9	1.1	1.0	1.0	0.9	0.8	1.6	0.8	0.8	0.5	0.4	0.4	0.4	0.3
16	0.8	1.0	0.9	1.0	0.9	0.8	1.3	0.7	0.6	0.3	0.4	0.3	0.3	0.5
18	0.9	0.9	1.0	1.0	0.8	0.7	1.1	0.7	0.5	0.2	0.4	0.2	0.2	0.7
19	1.0	1.0	1.0	1.0	0.9	0.7	1.1	0.7	0.5	0.3	0.3	0.3	0.3	0.5



What Is the Role of Sequences Flanking the N-Terminus of PR?

1. The Gag Frame Encodes p1 and p6

- p6 contains the L domain (PTAPP) which is critical for virus release from the cell
- p6 is required for proper incorporation of Vpr into the virions as well as retention of pol proteins
- p6 associates with TRiC (chaperonin)

2. The Pol Frame Encodes a Transframe Protein (TFR)

TFR includes a conserved octapeptide (TFP) and p6*

- The TFP is a potent competitive inhibitor of PR in vitro
- p6* modulates PR activity

Figure 15

1040902474850

3. Contains Sequences and Structures Required for Frameshift

- Slippery heptamer sequence (U UUU UUA)
- Stem loop structure downstream of the frameshift site

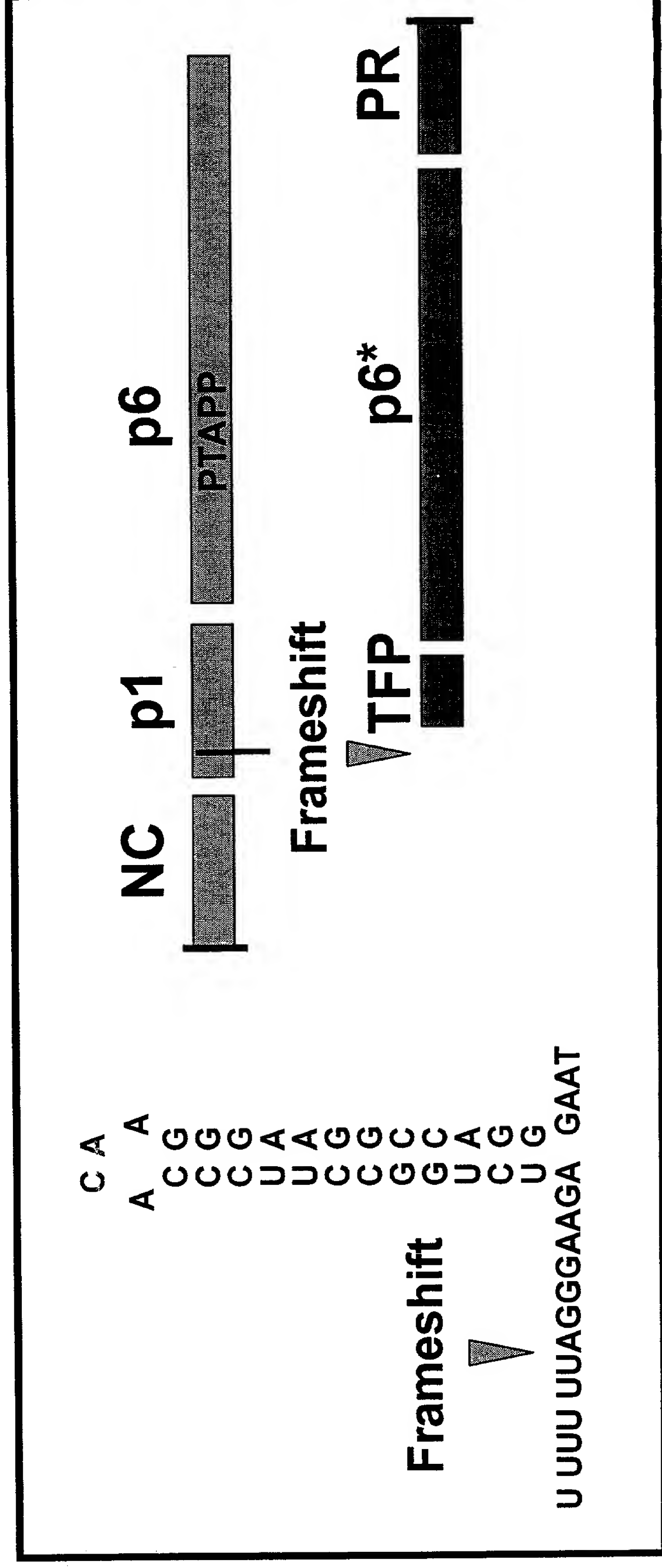


Figure 16

Genotypes of Patient-Derived Sequences

Gag p1 and p6

	ANFLGKIWP	SHKGRPGN	FLQSRPE	PTAPPE	ESFRFG	EET'TTP	SQKQEP	IDKELY	PLASLR	SLFGND	PSSQ
ISNAG
IIVSATKL
IIINTPTQL
IVGK

Transframe Protein

	FFREDLAF	FPQ	GKAREFS	SEQ	TRANSP	TRRE	LQWGRD	NNNS	LSEAGADR	QT	VSESP
ILSNNL
IIN	E..KL
III	P...PGPI
IVNT

* I to IV represent clones derived from patient sample pools that retained the HS to PI

Figure 17

RNA Sequencing and Protein Sequencing

C A
 A A
 C C G G A A G G C C A G G
 C C U U C C G G U C U
 U U U U U A G G G A A G A GAAT

Frameshift



C A
 A A
 C C U U C C G G U C U
 G G A A G G C C A G G
 U U U U U A G G G A A G A GAAT

I-

C A
 A A
 C C G G A G G C C A G G
 C C U U C C G G U C U
 U U U U U A G G G A A A A

II-

C A
 A A
 C C G G A A G G C C A G G
 C C U U C C G G U C U
 U U U U U A G G G A A GAAT

III-

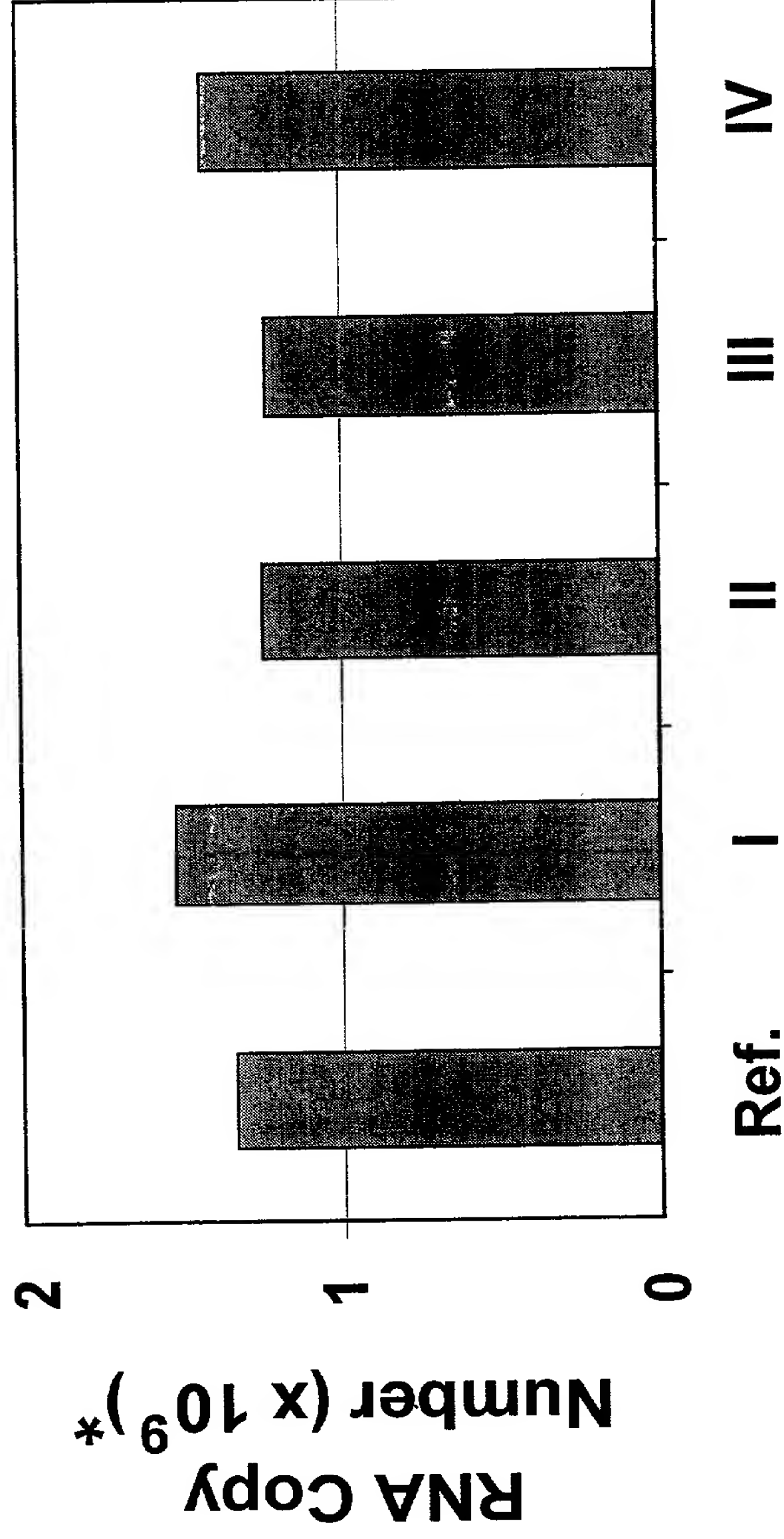
C
 C
 G G A A G G C C A G G
 C C U U C C G G U C U
 U U U U U A G G G A A G A GAAT

IV-

Figure 18

Virus Release from the Cell

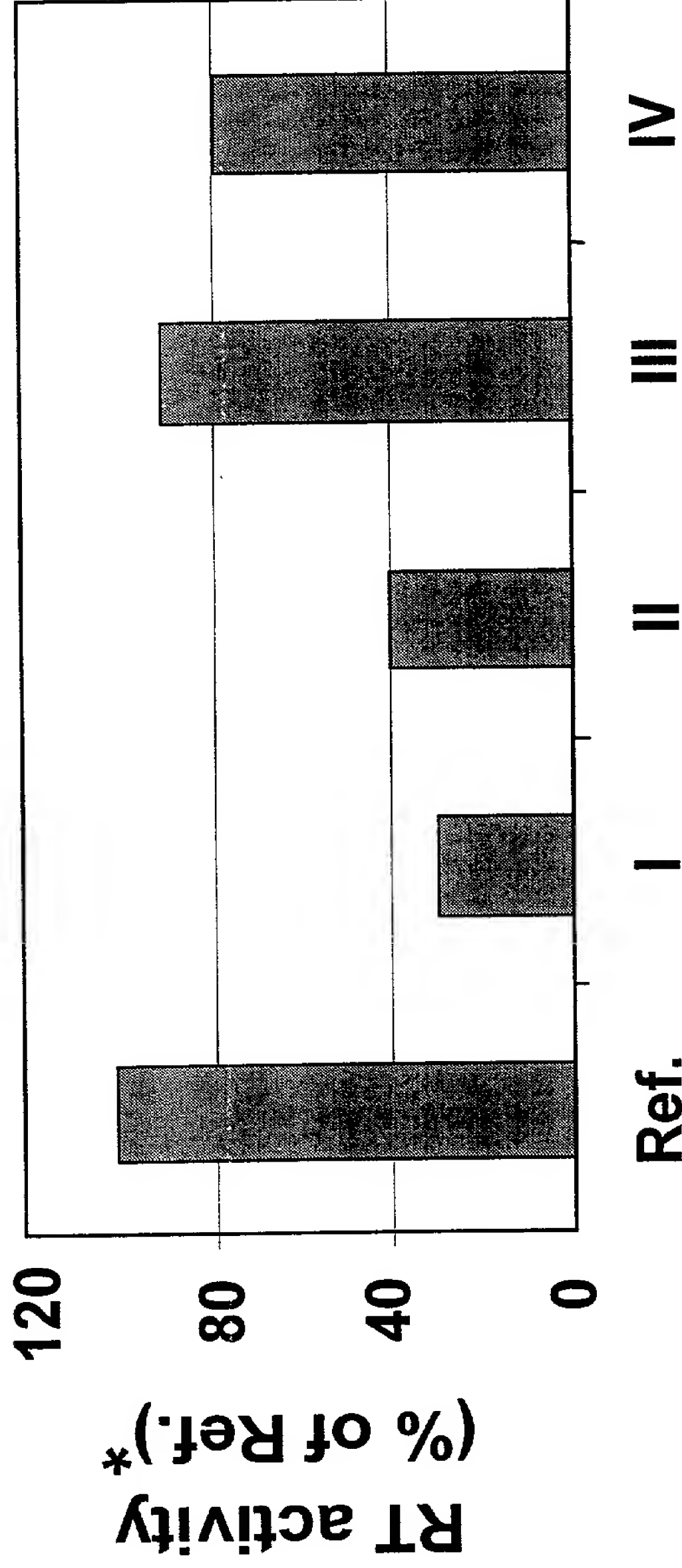
Quantitation of the amount of viruses produced after transfection



*Determined by Real Time PCR (TaqMan)

Pol Incorporation and/or Processing

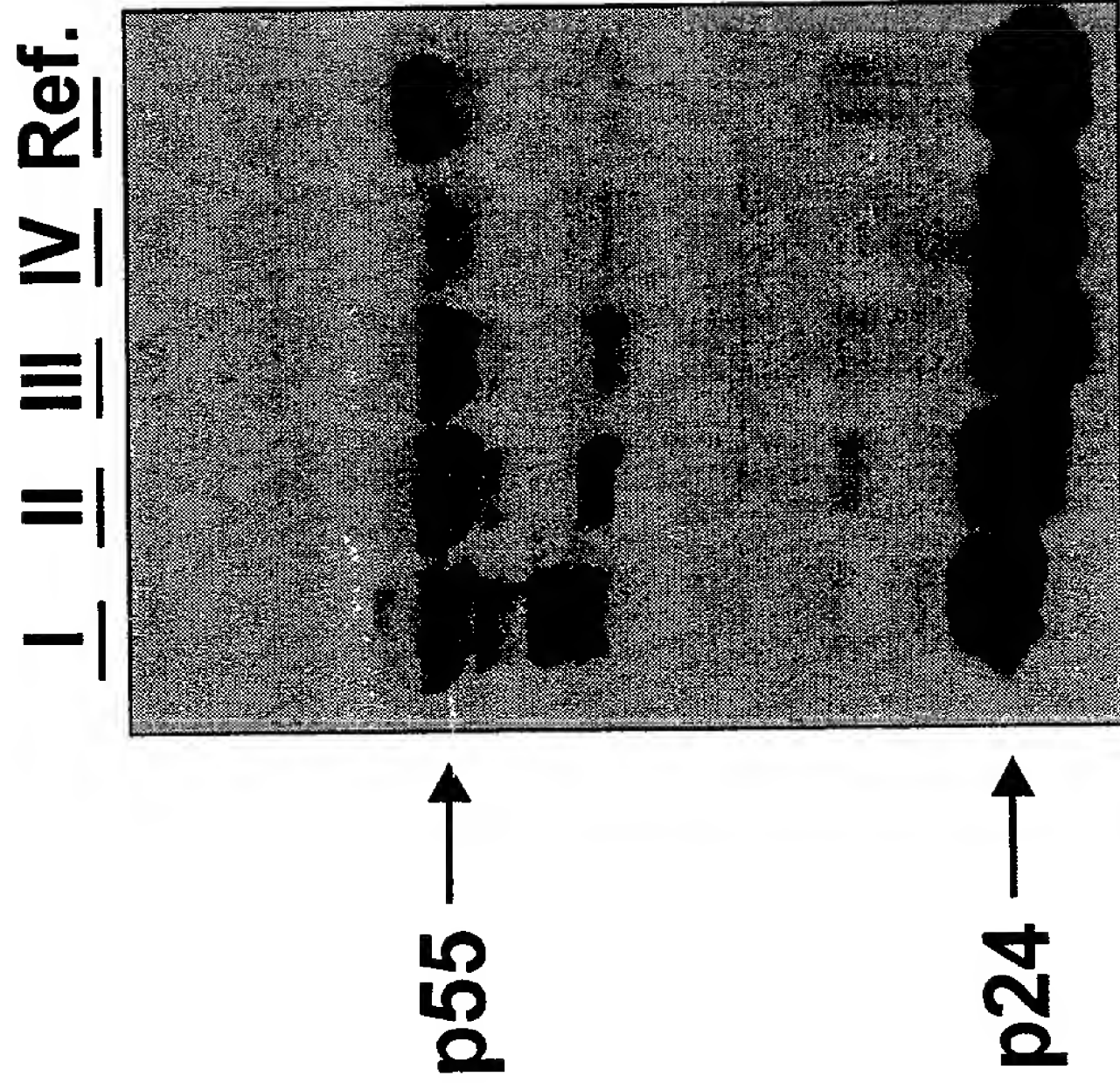
Quantitation of virion associated reverse transcriptase activity



* Determined by Real Time PCR (TaqMan)

Processing of Pr55Gag in Virions

Western Blot analysis using anti-p24 antibodies

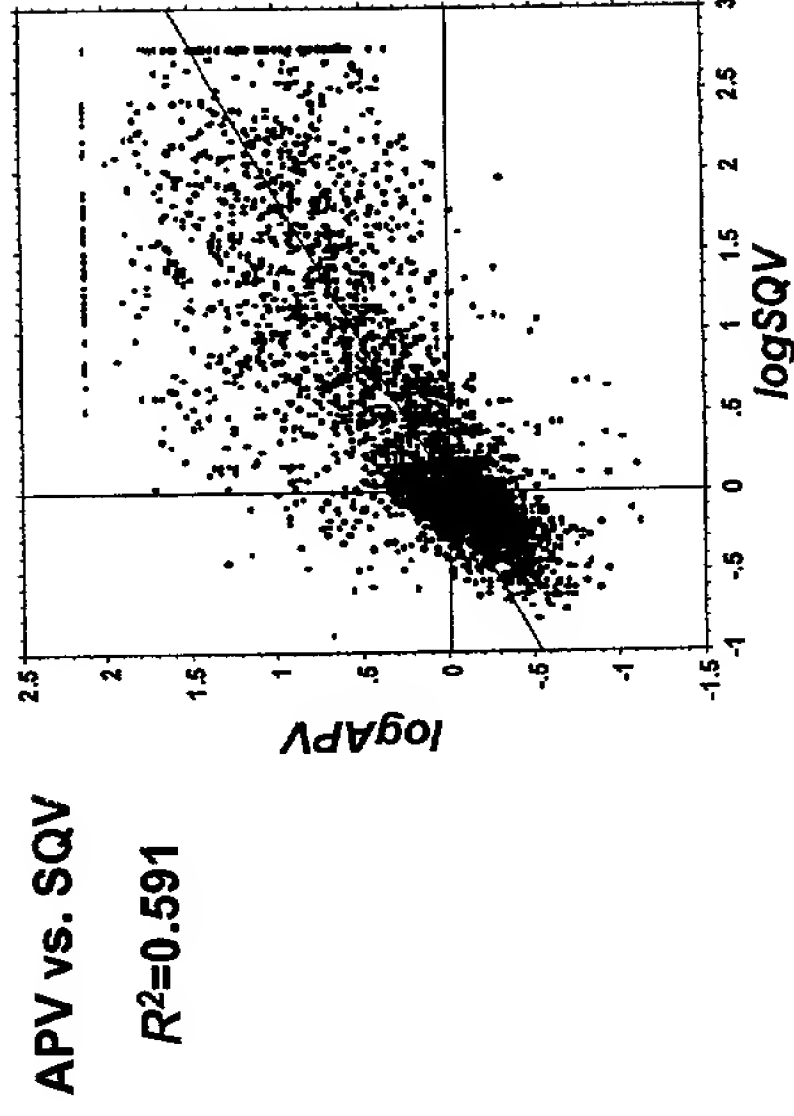
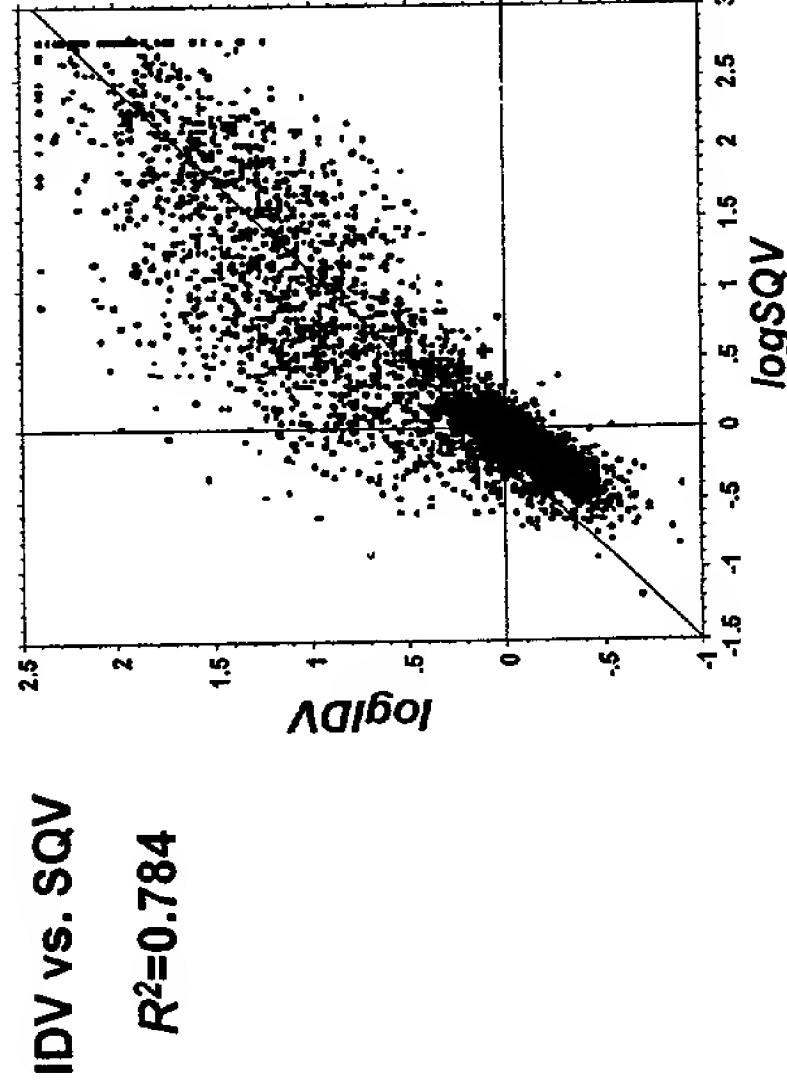
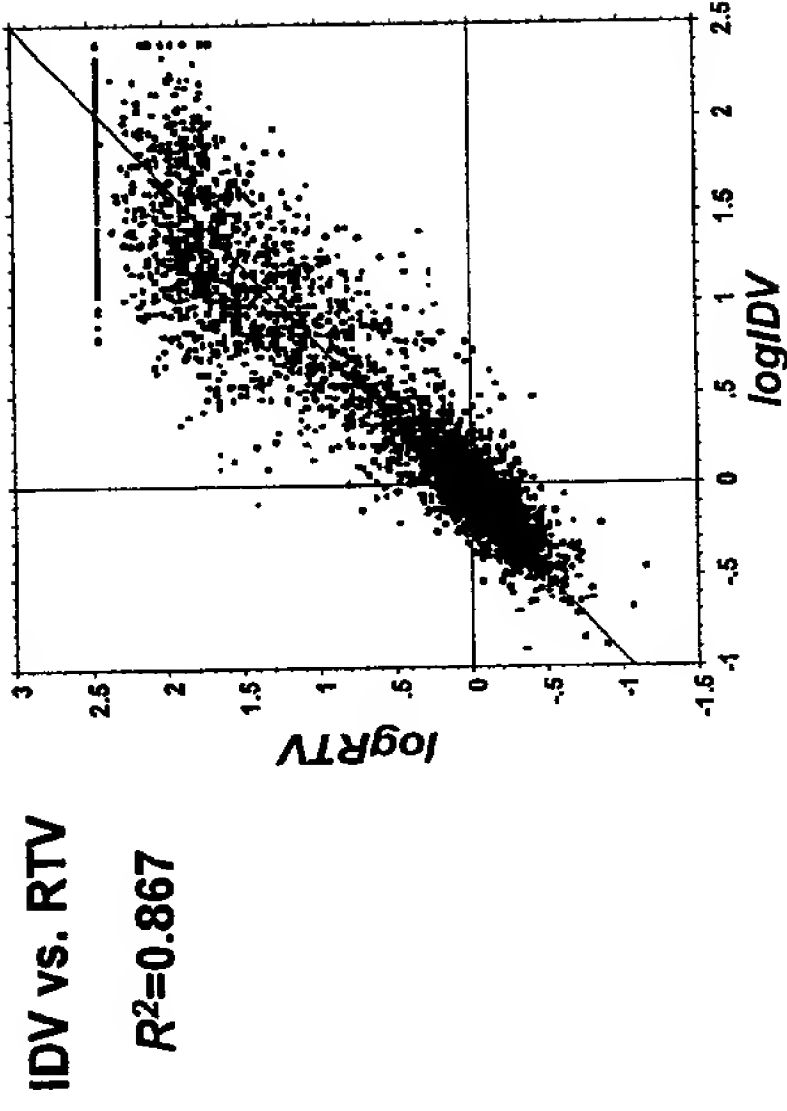
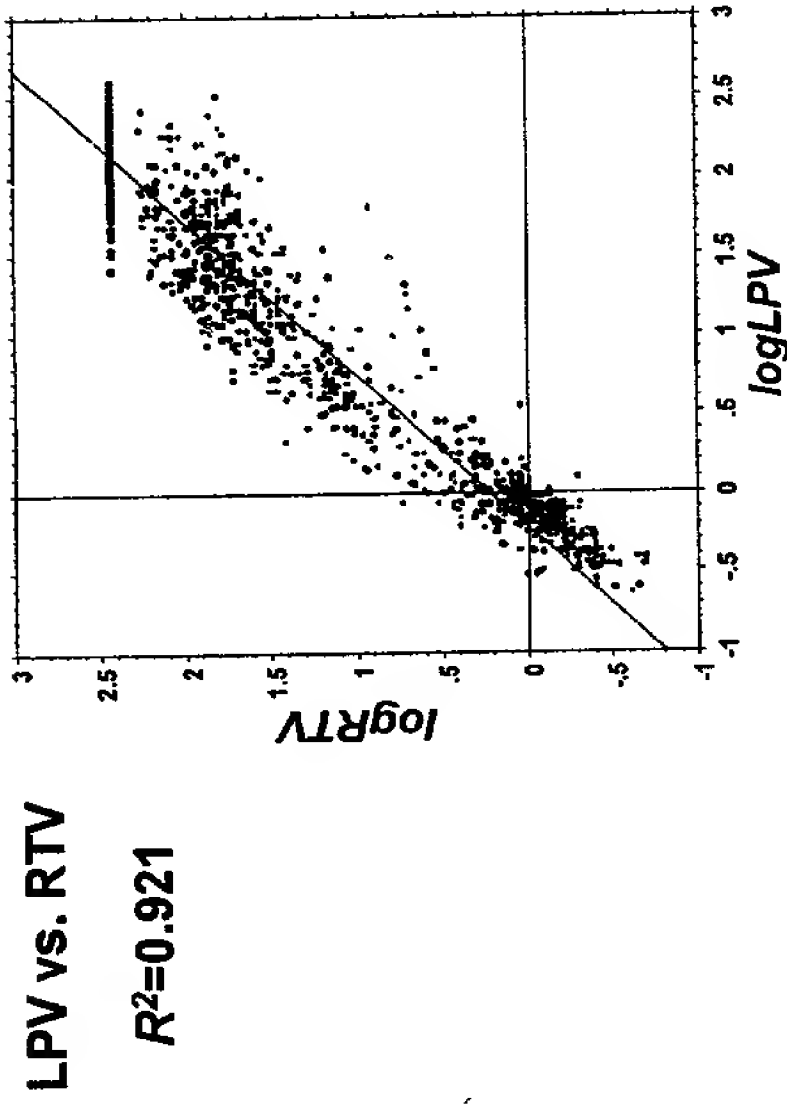


Conclusions

- HS to PIs is associated with decreased viral fitness
- In 25% of the cases analyzed in this study, the HS to PIs and decreased replication capacity was attributed to mutations in gag sequences flanking the N-terminus of PR
- Genotypic analysis revealed several unusual polymorphisms in p1-p6/TFP-p6* sequences
- Recombinant viruses carrying only the C-terminal gag sequences from patient isolates that retained the HS phenotype are released efficiently from the cell. However, analysis of the virus associated RT and PR activities suggest maturation defects

Figure 22

For 2020-2021 season



These plots are examples of pairwise analysis of the extent of cross-resistance between pairs of PIs. The fold-change in IC50 vs. reference (NL4-3) of 1042 (RTV-LPV) to >3600 (other pairs) patient samples were determined using the PhenoSense assay.

**R² values
(sorted by drug)**

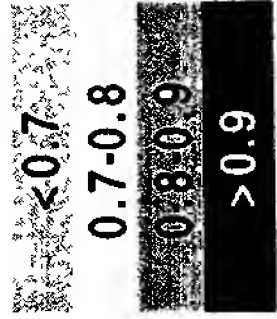
PI 1	PI 2	R ²
APV	IDV	0.675
APV	LPV	0.777
APV	NFV	0.544
APV	RTV	0.737
APV	SQV	0.591
IDV	LPV	0.774
IDV	NFV	0.774
IDV	NFV	0.925
IDV	RTV	0.777
IDV	SQV	0.784
NFV	LPV	0.757
NFV	RTV	0.696
NFV	RTV	0.691
NFV	SQV	0.691
NFV	SQV	0.691
RTV	LPV	0.921
RTV	SQV	0.740
RTV	SQV	0.591
SQV	LPV	0.678

**R² values
(sorted by R²)**

PI 1	PI 2	R ²
IDV	NFV	0.925
RTV	LPV	0.921
RTV	SQV	0.777
NFV	RTV	0.777
IDV	RTV	0.777
IDV	LPV	0.774
NFV	SQV	0.774
IDV	SQV	0.784
APV	LPV	0.777
IDV	NFV	0.774
NFV	LPV	0.757
RTV	SQV	0.740
APV	RTV	0.737
NFV	RTV	0.696
NFV	SQV	0.691
SQV	LPV	0.678
APV	IDV	0.675
APV	SQV	0.591
APV	NFV	0.544

R² values for pairwise comparisons (all samples)

	APV	IDV	LPV	NFV	RTV	SQV
APV	1	0.675	0.777	0.544	0.737	0.591
IDV	0.675	1	0.774	0.774	0.737	0.784
LPV	0.777	0.774	1	0.757	0.921	0.678
NFV	0.544	0.774	0.757	1	0.696	0.691
RTV	0.737	0.737	0.921	0.696	1	0.740
SQV	0.591	0.784	0.678	0.691	0.740	1



* Excluding viruses with D30N (see Fig.4)
 ** Excluding viruses with V82AFST (see Fig.5)

Correlation Coefficients (R^2) for all pairwise comparisons between PIs. After separating the D30N viruses in NFV comparisons (*) it can be seen that IDV, LPV, NFV and RTV have high levels of cross-resistance with each other, but that APV and SQV tend to be less cross-resistant. Removal of viruses with V82A, F, S, or T also reveals high level of cross-resistance between RTV and SQV.

**R² values
(sorted by drug)**

PI 1	PI 2	R ²
APV	IDV	0.675
APV	LPV	0.777
APV	NFV	0.544
APV	RTV	0.737
APV	SQV	0.591
IDV	LPV	0.849
IDV	NFV	0.774
IDV	NFV	0.925 *
IDV	RTV	0.867
IDV	SQV	0.784
NFV	LPV	0.757
NFV	RTV	0.696
NFV	RTV	0.873 *
NFV	SQV	0.691
NFV	SQV	0.801 *
RTV	LPV	0.921
RTV	SQV	0.740
RTV	SQV	0.880 **
SQV	LPV	0.678

**R² values
(sorted by R²)**

PI 1	PI 2	R ²
IDV	NFV	0.925 *
RTV	LPV	0.921
RTV	SQV	0.880 **
NFV	RTV	0.873 *
IDV	RTV	0.867
IDV	LPV	0.849
NFV	SQV	0.801 *
IDV	SQV	0.784
APV	LPV	0.777
IDV	NFV	0.774
NFV	LPV	0.757
RTV	SQV	0.740
APV	RTV	0.737
NFV	RTV	0.696
NFV	SQV	0.691
SQV	LPV	0.678
APV	IDV	0.675
APV	SQV	0.591
APV	NFV	0.544

R² values for pairwise comparisons (all samples)

	APV	IDV	LPV	NFV	RTV	SQV
APV	1	0.675	0.777	0.544	0.737	0.591
IDV	0.675	1	0.849	0.774	0.867	0.784
LPV	0.777	0.849	1	0.757	0.921	0.678
NFV	0.544	0.774	0.757	1	0.696	0.691
RTV	0.737	0.867	0.921	0.696	1	0.740
SQV	0.591	0.784	0.678	0.691	0.740	1

<0.7

0.7-0.8

0.8-0.9

>0.9

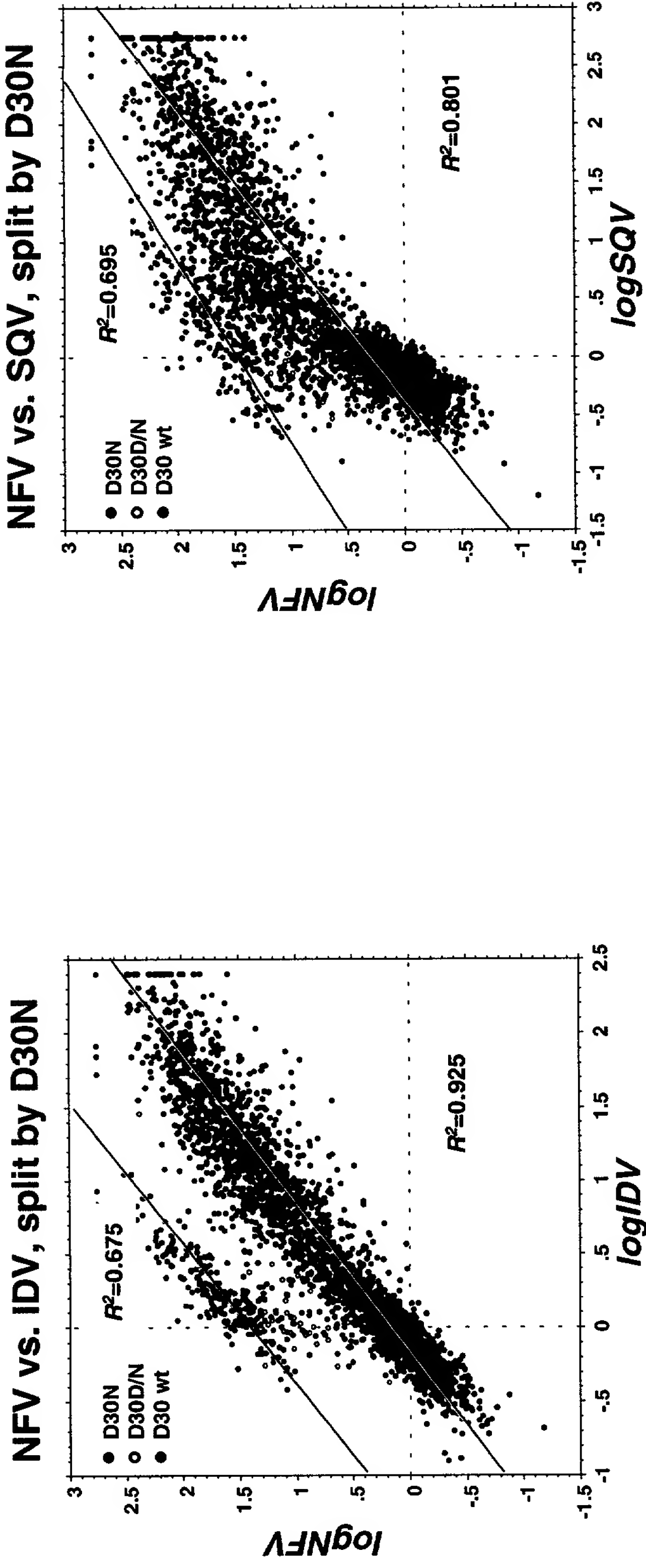
* Excluding viruses with D30N (see Fig.4)

** Excluding viruses with V82AFST (see Fig.5)

Correlation Coefficients (R²) for all pairwise comparisons between PIs. After separating the D30N viruses in NFV comparisons (*) it can be seen that IDV, LPV, NFV and RTV have high levels of cross-resistance with each other, but that APV and SQV tend to be less cross-resistant. Removal of viruses with V82A, F, S, or T also reveals high level of cross-resistance between RTV and SQV.

For SQV

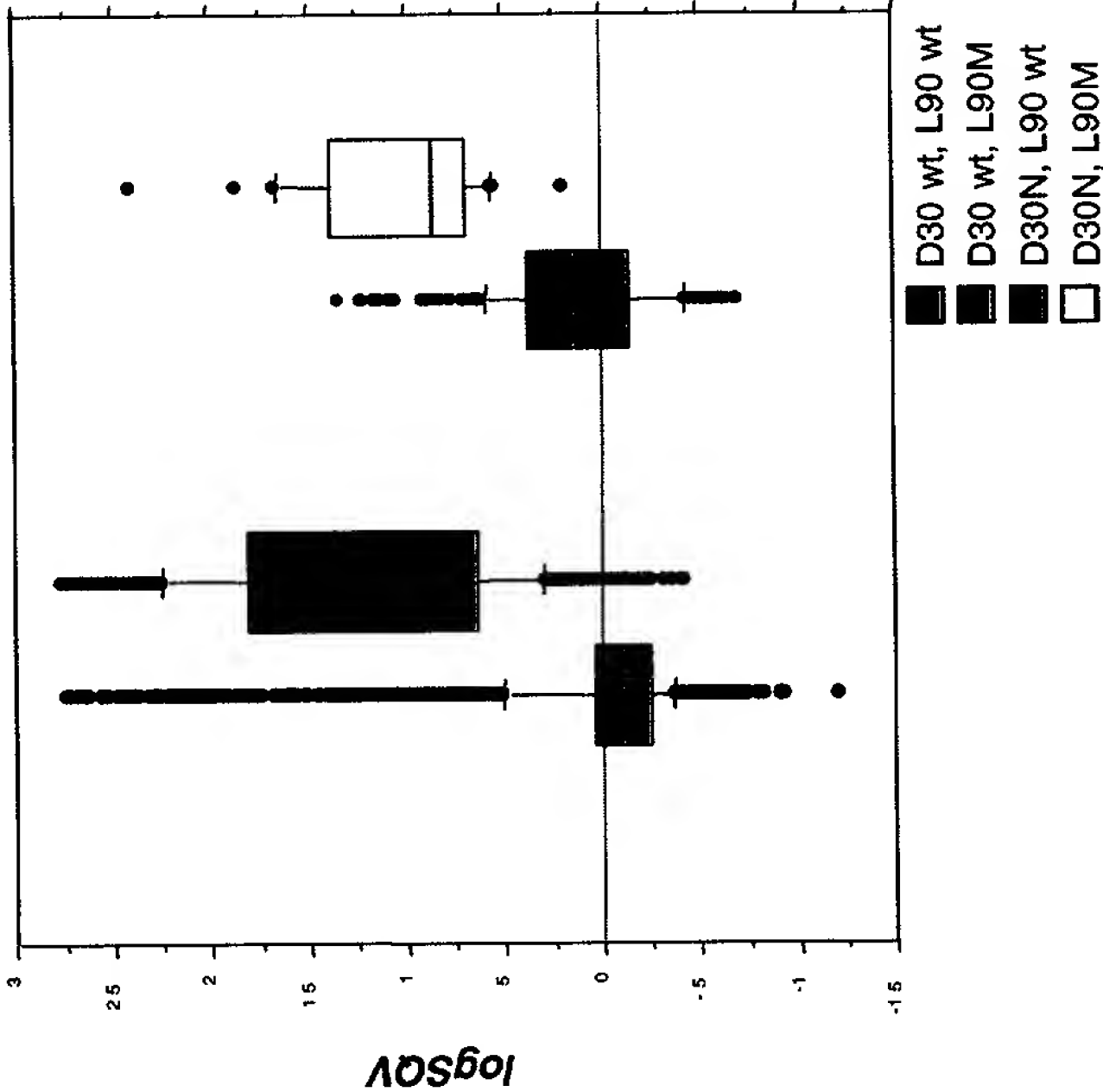
Figure 25



Scatter plots showing the separation of virus populations based on D30N, for IDV and NFV, or, SQV and NFV. There is still cross-resistance to IDV or SQV in D30N-containing viruses, albeit only at high levels of NFV resistance. These viruses tend to have the combination of D30N, N88D, and L90M (see next slide) The correlation between NFV and IDV in the absence of D30N is particularly striking.

Figure 26

SQV fold change +/- D30N, L90M



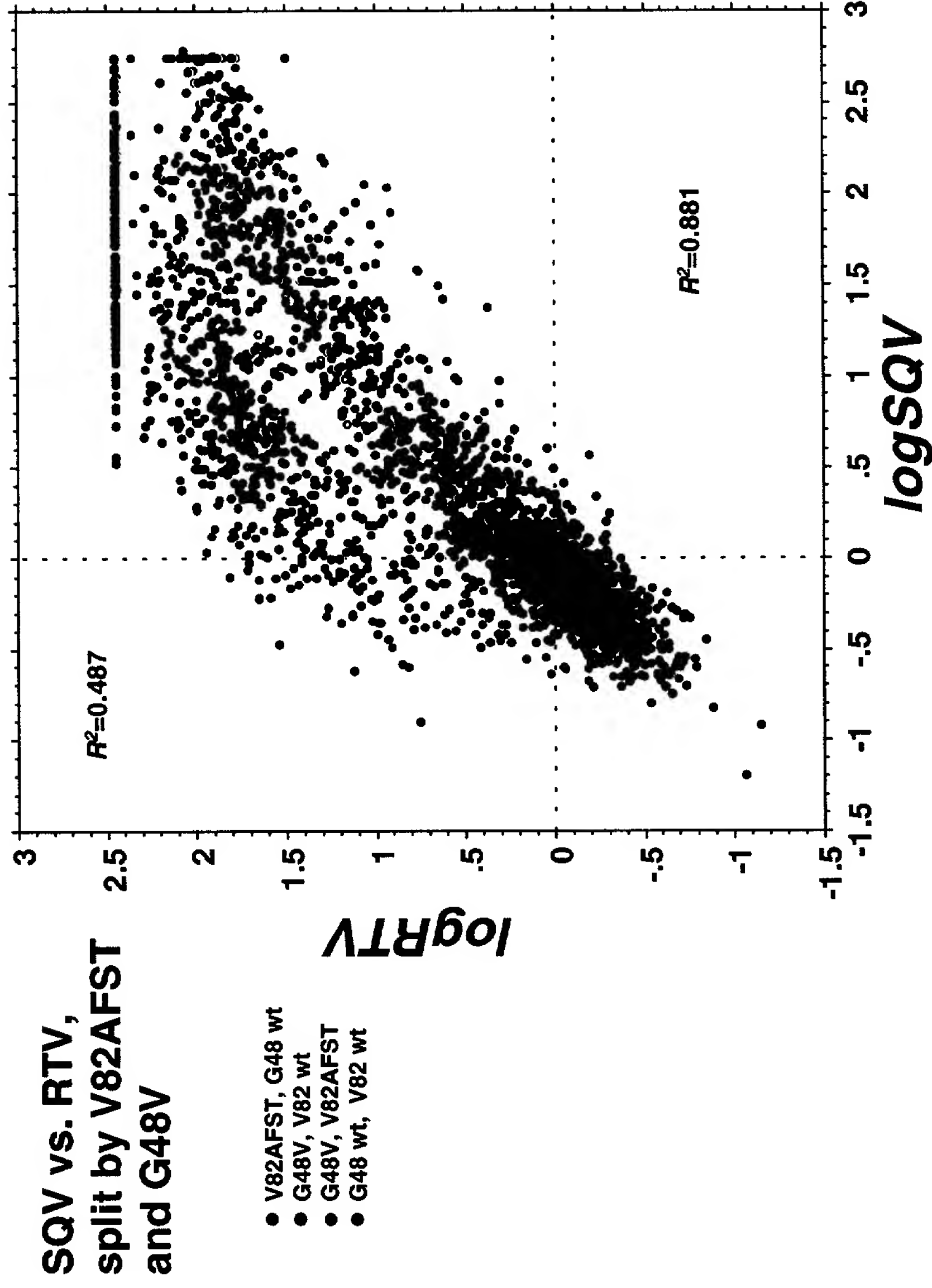
Phenotypes of samples containing D30N, and/or L90M, from the database (boxes contain a bar at the median and represent the 25th to 75th percentiles; the error bars represent the 10th and 90th percentiles; and the dots are the outliers.

D30N/N88D/L90M: Patient samples

PR genotype (resistance-associated mutations)	Fold-change in IC ₅₀ vs. reference				
	AMP	IDV	NFV	RTV	SQV
L10L/V, D30N, L33L/F, M36I, L63P, A71T, N88D, L90M	1.9	160.4	10.2	10.2	10.2
D30N, L63P, V77I, N88D, L90M	1.3	74.2	10.2	10.2	10.2
D30N, M36I, L63P, A71T, N88D, L90M	1.1	124.0	10.2	10.2	10.2
D30N, L63P, V77I, N88D, L90M	2.0	57.0	10.2	10.2	10.2
L10F, D30N, L33F, I54L, L63P, A71V, V77I, N88D, L90M	11.4	1.1	108.8	10.2	10.2
L10F/Y, D30N, I54L, L63P, A71T, V77I, N88D, L90M	0.4	1.3	171.4	10.2	10.2
D30N, L63P, V77I, N88D, L90M	2.3	217.5	10.2	10.2	10.2
L10F, D30N, L63P, A71T, V77I, N88D, L90M	2.3	140.1	10.2	10.2	10.2
L10L/R, D30N, M36I, I54I/L, L63P, A71V, N88D, L90M	1.5	218.5	10.2	10.2	10.2
D30N, M36I, I54V, L63P, A71V, N88D, L90M	2.3	>550	35.0	72.0	72.0
K20K/R, D30N, M36I, F53F/L, I54V, L63P, A71V, N88D, L90M	1.2	1.7	46.9	10.2	10.2
L10L/F, I13I/V, L19T, D30N, R41K, L63P, N88D, L90M	1.0	2.3	66.8	10.2	10.2
D30N, L63P, V77I, N88D, L90M	27.6	>550	31.2	45.3	45.3
L10F, K20T, D30N, L33F, M36I, M46M/I, I54L, L63P, A71V, V77I, N88D, L90M	1.3	1.3	35.7	10.2	10.2
D30N, L33F, L63P, A71A/T, N88D, L90M	1.5	73.7	10.2	10.2	10.2
D30N, L63P, V77I, N88D, L90M	2.2	12.0	140.4	27.0	45.8
D30N, M36I, I54V, L63P, A71V, N88D, L90M	>130	>250	>550	>275	257.5
L10F, K20R, D30N, V32V/I, L33L/F/I, M36I, M46I, I47I/V, I54I/A/M/T/V, L63P, A71V, V82V/A, N88D, L90M	>130	>250	>550	>275	257.5

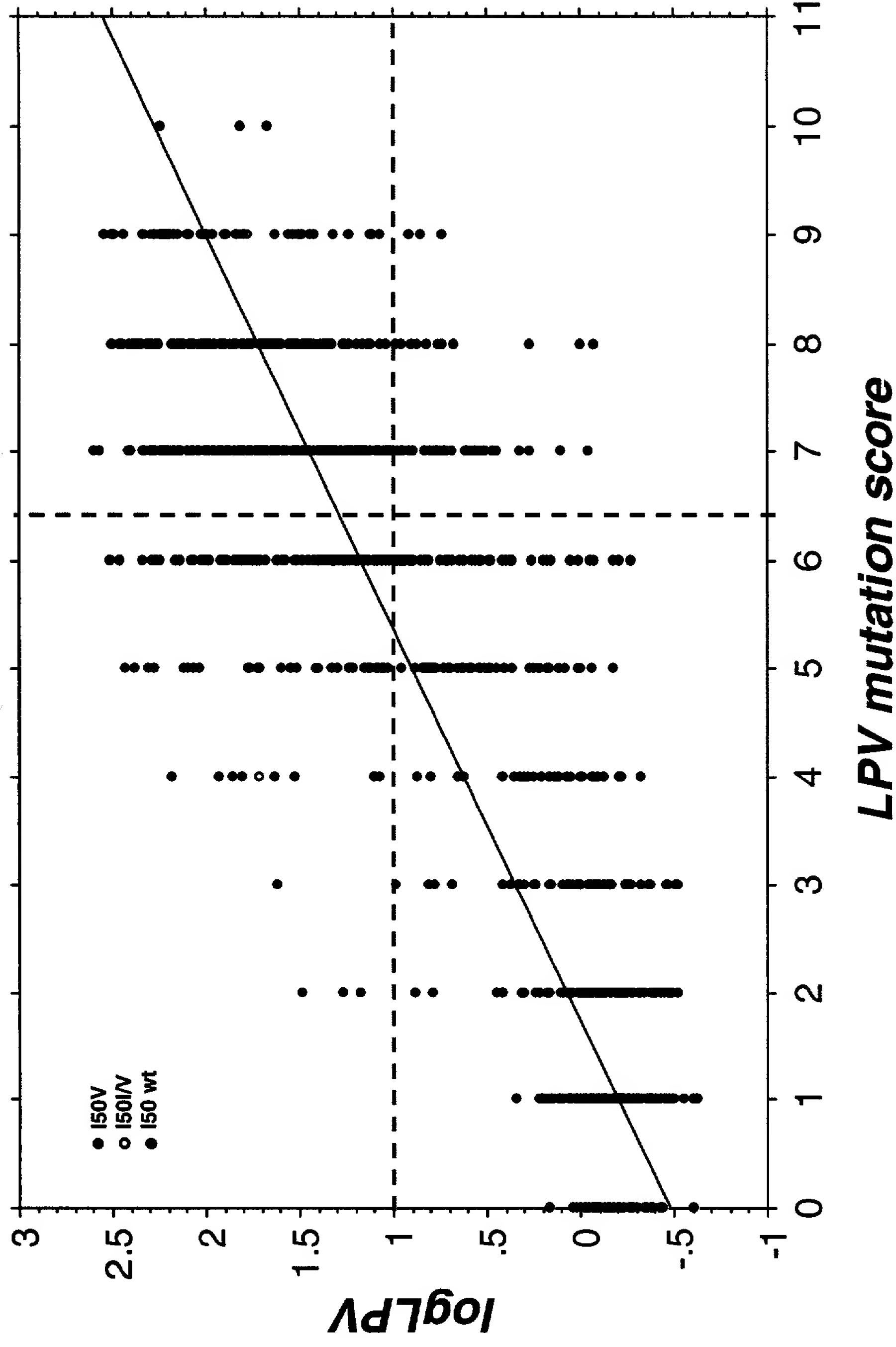
Phenotypes of samples containing D30N, N88D, and L90M. There are no mixtures detected at these sites, indicating that the mutations are linked. All have reduced susceptibility (>2.5-fold change in IC50) to NFV and SQV.

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Scatter plot showing the separation of virus populations based on V82A, F, S, or T, for RTV and SQV. There is greater cross-resistance between RTV and SQV in viruses lacking position 82 mutations than in the population as a whole. Viruses with V82A, F, S, or T have more resistance to RTV than to SQV, unless they also have G48V (black dots)

Figure 28



Scatter plot showing the relationship between LPV susceptibility and LPV mutations score (number of mutations at positions 10, 20, 24, 46, 53, 54, 63, 71, 82, 84 and 90). Mixtures were counted as mutant and all variants at each position were considered. Clinically relevant cut points for phenotype (10-fold) and genotype (6 mutations) have been previously defined for LPV. The “false negatives” (LPV resistant with <6 mutations) contain several viruses with the I50V mutation (red dots).